Carefully estimating the incidence of natalizumab-associated PML

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Abstract

We show that the quarterly updates about the risk of PML during natalizumab therapy, while in principle helpful, underestimate the real incidences systematically and significantly. Calculating the PML incidences using an appropriate method and on realistic assumptions, we obtain estimates that are up to 80% higher. In fact, with the recent paper [Plavina et al 2014], our approximate incidences are up to ten times as high. The present article describes the shortcomings of the methods used in [Bloomgren et al 2012] and by Plavina et al for computing incidences, and demonstrates how to properly estimate the true (prospective) risk of developing PML during natalizumab treatment. One application is that the newest data concerning the advances in risk-mitigation through the extension of dosing intervals, although characterised as not quite statistically significant, are in fact significant. Lastly, we discuss why the established risk-stratification algorithms, even on assessing the PML incidences correctly, are no longer state-of-the-art; in the light of all the progress that has been made so far, already today it is possible to reliably identify over 95% of patients in whom (a personalised regimen of) natalizumab should be very safe.

Results

The following shows the incidence of PML for JCV-positive natalizumab-treated patients according to [TY-PAN-0597(17) 2014] (see [Bloomgren et al 2012] for the method) vs our estimates:

constellation	(worldwide) PML risk as stated in the latest quarterly report	realistic PML risk estimate
mo. 25–48, prior IS use	1 : 89	1 : 51
mo. 25–48, no prior IS use	1 : 189	1 : 135
mo. 49–72, no prior IS use	1 : 164	1 : 91

The next table further specialises to individuals without previous immunosuppressive therapies, giving PML incidence estimates as a function of JCV titre, adapted from [Plavina et al 2014]:

	PML risk estimate for mo. 25–48		PML risk estimate for mo. 49–72	
JCV antibody index	[Plavina et al 2014]	realistic	[Plavina et al 2014]	realistic
≤ 0.9	1 : 3333	1 : 2000	1 : 2500	1 : 1250
> 0.9 and ≤ 1.1	1 : 1429	1 : 189	1 : 1429	1 : 127
> 1.1 and ≤ 1.3	1 : 1000	1 : 149	1 : 833	1 : 100
> 1.3 and ≤ 1.5	1 : 833	1 : 333	1 : 769	1 : 227
> 1.5	1 : 123	1 : 88	1 : 118	1 : 59

Straightaway, the reader notices that patients with levels between 1.3 and 1.5 appear to have a lower risk than patients in the range 1.1–1.3. This does of course not mean that it is in principle not reasonable to use multiple thresholds; however, at this time, there is clearly not enough data to meaningfully differentiate between individuals as finely as Plavina et al attempted to.

§1. Introduction

Whenever the risk of PML during therapy with natalizumab in patients with multiple sclerosis is discussed, many a time, one source is referred to [Bloomgren et al 2012]. In this paper, the authors develop a *risk-factor algorithm*, sometimes also called *risk-stratification algorithm*, or just plain *risk algorithm*, in order to assist patients and their physicians in making treatment decisions. Unfortunately, the method employed by Bloomgren et al for quantifying the risks is inadequate, because according to the resulting estimates, natalizumab-associated PML should occur

a lot more seldom than is actually the case.

Doubts about the suitability of this particular way of assessing incidences have been raised by others. Roughly nine months ago, Gavin Giovannoni wondered in a *tweet* [Giovannoni 2014a]: '[Are] we underestimating the PML risk?' Moreover, several weeks earlier already, Giovannoni had nicely summarised the state of affairs on the *Barts MS Research Blog* [Giovannoni 2014b]: 'What is also not captured in these figures is the fact that the denominator is changing; the number of MSers at risk of PML staying on the drug goes down with time therefore the number of cases (numerator) are being divided by a changing denominator. For this reason I suspect that the actual risk after 2 years of treatment is much higher than that reported.' Below (§§3 and 5) we will see that Giovannoni is exactly right.

The importance of knowing the true PML incidences was stressed a long time ago. An online magazine covering the 13th case of natalizumab-associated PML quotes Ralf Gold [SPON 2009]: 'We want to keep [Tysabri], but need to know of new cases. Safety takes precedence over the financial interests of the drugmaker.' A key question directly related is that of how to best communicate data on the risk of PML, as highlighted in a lecture delivered by Alice Hughes [Hughes 2011, slides 10–11]: 'FDA believes that presenting PML incidence for discrete intervals of treatment ... will aid healthcare professionals in discussing the risk of PML with their patients ... We decided that the [interval] presentation ... is more clinically useful ... in discussing risk over time with patients.' This last point is precisely why this topic is highly relevant: for people to be able to make an informed decision, it is obviously essential for them to understand how their risks change over time.

Given the fashion PML incidences are disseminated, it should be clear what inferences to draw from them. To avoid confusion, the following example, again taken from a blog post of Giovannoni's, shows how to interpret the risk-factor algorithm [Giovannoni 2014b]: 'So if you are JCV positive, have had no prior exposure to immunosuppression and have been on the drug 25 months ... your risk in the next 24 months is 1 in 189.' Normally, this bit of information would be very helpful, since an individual can then decide if 1 in 189 is an acceptable level of risk to them. The thing is, an accurate estimate of the PML incidence in this context is approximately 1 in 135.

Matters get strikingly worse if one in addition assumes that such a patient's JCV antibody index is, say, 1.063. For, as stated in [Plavina et al 2014, table 2], that person's risk of PML is then only 0.7‰ (1 in 1429) during months 25–48 of natalizumab therapy, but in actual fact, their risk is about 1 in 204—around seven times as high. The root cause of this rather enormous inconsistency is that when estimating the PML incidences in individuals without previous immunosuppressant use and whose JCV titre levels do not exceed 1.1, Plavina and coauthors also include all JCV-positive patients having a titre level less than or equal to 0.9.[1] However, this completely distorts the picture, because as per their own dataset, five out of six non-PML patients in the former category are in the latter as well, but merely two out of six PML cases.

This work is organised as follows. We will start out by describing three heuristic observations as to why Biogen Idec's approach to computing risks is not adequate. One heuristic will be that the PML incidence estimates from the STRATA study are much higher than those obtained using post-marketing data. As a by-product, the findings from STRATA further show that the concept of a *drug holiday* has merit after all, ie, that a planned treatment interruption is indeed conducive to natalizumab risk-mitigation. Next we will explain in detail the sources of the errors in the PML statistics by Biogen Idec, and give a comprehensive account of how to correctly estimate the incidences with the ordinary risk-factor algorithm (from [Bloomgren et al 2012]). Along the way, we will provide evidence that risk-stratification does work, a hypothesis that was challenged [Cutter and Stüve 2014]. We shall then refine the approximations of the risk in JCV-positive people not having previously been treated with immunosuppressants, ie, we will demonstrate how to derive realistic PML incidence estimates for the enhanced risk algorithm introduced in [Plavina et al 2014].

A corollary to our results is that it is in fact attainable for a natalizumab-treated person to significantly lighten their burden of therapy, by extending the dosing interval to, eg, six or seven weeks, while maintaining the efficacy of the standard (ie, monthly) infusion schedule. This has been established in [Ryerson et al 2014], which collects 861 years of exposure—as yet without any PML cases—among JCV-seropositive individuals realising the idea of stretching the distances between cycles. Very briefly, although the study authors say that 1248 years are necessary to reach statistical significance at the 5% level, via the risk estimates from this article, we will see that the experience gained thus far already suffices to attest that this strategy is potent when it comes to decreasing the incidence of PML. What is more, utilising elongated intervals seems to be indicated particularly in people with lower weight (less than 70 kg), as has been in the air for some time [Foley et al 2012, Foley 2013]; for updated information, see [Foley et al 2014]. In fact, even the FDA now apparently thinks that the regular natalizumab dosage may be too high [MSology 2014].

We will close by reviewing two innovative parameters strongly affecting the odds of PML on natalizumab: the proportion of CD62L-expressing $CD4^+$ T-lymphocytes in blood [Schwab et al 2013], and the presence or absence of lipid-specific IgM bands in CSF [Villar et al 2015]. The

[1] When Plavina et al assess the risks for patients with index at most 0.9, they (rightly) exclude everyone JCV-negative. Similarly, when determining the risks in those of index up to 1.1 (1.3, 1.5), all individuals of index up to 0.9 (respectively 1.1, 1.3) should be excluded to get the most precise possible estimates.

combined predictive power of these two biomarkers is expected to dramatically ameliorate the situation for over 90% of individuals left at considerable risk by the traditional algorithms—almost half of all long-term natalizumab users—namely those with a JCV index greater than 0.9 or who are JCV-positive and have had prior immunosuppression. Explicitly, after receiving natalizumab for a period of 18–24 months, such people are at the moment frequently (but most usually needlessly so) *deriskified*, ie, switched to a different pharmacological therapy. Fortunately, on incorporating the two above-mentioned novel markers into routine medical practice, risk-stratification can be made much more specific, so that most patients will be able to stay on treatment, knowing that their annual PML risk is less than 1 in 1000, or even 1 in 2000, though possibly requiring a little individualisation of the dose. To sum up, with the essentially only serious complication linked with the prolonged administration of natalizumab shortly more or less eliminated, the future of this highly-effective MS drug is very bright.

§2. Heuristic reasons suggesting that there is a flaw in the Biogen Idec methodology

In this section, we will bring forward three heuristic arguments that all lead to the conclusion that something must be wrong with the manner Biogen Idec estimate the PML incidences. For our first heuristic, we shall look at the 95% confidence intervals computed for the risk-factor algorithm back in March 2011. Our second heuristic involves examining the incidences from the US product label of Tysabri. The third heuristic goes by comparing the estimates of the frequency of PML in the STRATA trial with those for the post-marketing setting. Incidentally, the results from said study also (positively) answer the question if drug holidays are a useful means of lowering an individual patient's PML risk, ie, whether or not the 'infusion counter' is actually reset when natalizumab is restarted after a sufficiently long period of abstinence.

Confidence intervals. One heuristic viewpoint that facilitates spotting that something is probably not entirely right with the way the PML incidences are estimated is to consider the four 95% confidence intervals calculated for the three-factor risk-stratification algorithm in early March 2011 (about the time this algorithm was initially popularised); a quick inspection reveals that two of them do not contain the most recent estimates. For the 95% confidence intervals as of 4th March 2011 concerning the PML incidence in JCV-positive natalizumab-treated patients without prior exposure to an immunosuppressant are 0.19–0.60‰ for months 1–24 and 1.80–3.40‰ for months 25–48 [Kappos et al 2011a, figure 3]. However, according to the latest (global) data, of 5th March 2013, the PML incidence in patients with the aforementioned combination of risk factors is 0.7‰ for months 1–24 and 5.3‰ for months 25–48 [TY-PAN-0597(17) 2014].[2] Observe further that the estimated incidence in JCV-seropositive patients with former immunosuppressive treatment for months 25–48 as of 5th March 2013 (11.2‰) is barely inside the 95% confidence interval of two years earlier for this category (5.20–11.30‰).

Given four 95% confidence intervals, the chance of two or more not covering the actual values of the estimated population parameters is 1.4%. Additionally, in the case of two 'misses', with probability three-quarters, at least one of them should have occurred below the lower endpoint of the respective interval, which is not so—both confidence intervals in question underestimate the risks. This cuts the odds that these observations are purely random to 0.4%. The critical reader might object that this argument overlooks that the exact PML incidences are unknown and could be smaller than their current estimates. However, the two concerned figures, which can be assumed to converge to their true values over time as patient numbers grow, have only increased between updates, but never decreased (appendix A). Hence the real incidences, whatever they may be, are surely not smaller than the latest estimates.

The US Tysabri product label. Another approach to heuristically see that something is likely not correct with the PML incidences is supplied by way of the last alteration to the Tysabri package insert in America. Following this change, the product label now shows the incidences in JCV-positive patients for up to six years of treatment, according to prior or no prior use of immunosuppressive drugs [Tysabri PI 2013, table 1]. For months 25–48, the incidences are specified as 13‰ respectively 3‰. So in the US, if a patient has had immunosuppressive pretherapy, their risk for the third and fourth years of natalizumab treatment is roughly fourfold that of a patient never exposed to immunosuppressants. On the other hand, for months 49–72, the difference is much smaller—patients with previous immunosuppressive therapy are at a 9‰ chance of getting PML vs 7‰ with people not having received such medication. This means that whether immunosuppression has taken place seems to significantly influence the PML risk only during the first three or four years of natalizumab but not beyond, which is surprising. Even more astonishing is perhaps the fact that in individuals who are not sometime immunosuppressant users, the risk of PML for months 49–72 is over twice that for months 25–48, whereas in those with prior exposure to an immunosuppressant, the risk of developing PML during the former period is actually 30% lower compared to the latter.

The most probable explanation for these somewhat pe-

[2] The corresponding (ie, of March 2013) 95% confidence interval for the incidence of PML in the latter group is 4.4–6.2‰, which is considerably disjoint from, ie, pretty far from having overlap with, the original interval.

culiar findings, which are based on post-marketing data as of 3rd September 2013, is that there were simply not very many patients in the US with previous immunosuppressive therapy who had reached the fifth year of natalizumab treatment at the time these incidences were calculated.[3] As the discrepancies between the real PML incidences and those obtained with the Bloomgren method are especially large when patient numbers are small, it is quite likely that the risk of PML during months 49–72 in JCV-positive US patients having had immunosuppression is substantially higher than 9‰, certainly not lower than with the preceding 24 months, which would resolve the apparent contradictions.

Confidence intervals continued. Returning to the first heuristic, the change to the package insert of Tysabri in the US indirectly demonstrates that three (not just two) of the original four 95% confidence intervals with the PML risk-factor algorithm do not contain the true values of the parameters in question. Recall that we already noted that the latest estimate of the worldwide incidence of PML for months 25–48 of natalizumab treatment in JCV-positive patients with previous immunosuppressive therapy (11.2‰) is only just inside its 95% confidence interval of March 2011 (5.20–11.30‰). However, the updated Tysabri product label states that this incidence is 13‰. Appealing to what is sometimes referred to as the *EU/US paradox*—the fact that the incidence of natalizumab-associated PML in the EEA is significantly higher than in America [Hunt and Giovannoni 2012, p 29]—we deduce that the global incidence in this context must be greater still (than 13‰) and a fortiori outside the interval 5.20–11.30‰. Thus, three of the four 95% confidence intervals calculated for the risk-stratification algorithm in early March 2011 do not contain the true values of their estimated parameters, and in each of the three cases, the risk is in fact underestimated. The likelihood of all that being due to chance alone is 0.00007; equivalently, with 99.993% probability, the explanation for the in hindsight wrong predictions must be something else.

STRATA. Our third heuristic is that the incidence of PML—estimated in the style of Biogen Idec—in the cohort from the STRATA study (NCT00297232) is much higher than in the post-marketing setting, and actually, for months 49–72 of natalizumab giving, the STRATA incidence estimate is even significantly higher.[4] The following table shows the post-marketing incidences vs the incidences in STRATA as of August 2013 (using the data from [O'Connor et al 2014, figure e-2]):

	estimated PML incidence	
infusions	post-marketing	STRATA
1–24	1 : 1923	0
25–48	1 : 280	1 : 149
49–72	1 : 283	1 : 107

Table 1: Estimated PML incidences in natalizumab-treated patients in the post-marketing settings vs the STRATA study.

So although there were no cases of PML in the first two years of natalizumab therapy in STRATA among 1094 patients exposed, already for months 25–48, the estimated STRATA incidence is a good deal higher. With the next 24 months, the incidence in the STRATA trial is in fact significantly higher: out of 641 people with 49–72 natalizumab cycles, six developed PML (9.4‰); in comparison, in the post-marketing setting, the incidence in this constellation was 103 in 29 197 (3.5‰). That is, with individuals having had 49–72 infusions, PML appears to occur approximately 2.7 times as often in STRATA as in post-marketing, a difference that is statistically significant ($p = 0.0366$ [χ^2 test with Yates's correction]).

As always, one has to be cautious though—while JCV-positivity is greater in STRATA than what Bloomgren et al assume (67% vs 55%), the proportion of the prior use of immunosuppressive agents (7%) is considerably lesser [O'Connor et al 2014, p 81]. The former of course makes the gap regarding the frequency of PML between STRATA and post-marketing seem bigger than it really is; in contrast, the latter has the opposite effect. We shall now demonstrate how to properly take this into account, namely, by applying a correction factor; upon doing so, the distinction between STRATA and post-marketing will still be significant.

In the article [Bloomgren et al 2012], the authors suppose 18.7% previous immunosuppression for patients in the post-marketing setting with three or four years' natalizumab treatment. Moreover, according to their analysis, the risk that a JCV-positive person with prior exposure to immunosuppressants has during this period is 2.4 times the risk of somebody without pretherapies of this kind. To obtain a conservative correction factor, we will therefore assume that 15.5% of natalizumab users outside STRATA with 49–72 doses received immunosuppressive medicine at some point, and that these people have just double the risk. Hence our correction factor is

$$\frac{67\% \times (7\% \times 2 + 93\%)}{55\% \times (15.5\% \times 2 + 84.5\%)} = 1.129, \qquad (1)$$

and thus the 641 STRATA participants in question carry about the same risk as 724 patients (641×1.129) from post-marketing; even so, the six cases of PML in the STRATA trial in individuals with 49–72 natalizumab infusions are still significantly more than the 103 post-mar-

[3] Six months before, according to Biogen Idec's PML updates, there was 'insufficient data' to estimate the risk for months 49–72 in JCV-positive patients with prior IS treatment, despite these reports reflecting worldwide (vs US-only) experience.

[4] In this subsection, when we speak of months X–Y, what we technically mean is the period starting with infusion no. X up to and including infusion no. Y.

keting cases (p=0.0369 [one-tailed χ^2 test with Yates's correction] respectively p=0.0493 [one-tailed Fisher's exact test]).[5]

One might think that the reason for PML being commoner in STRATA is that the majority of subjects are based in the EEA/ROW [O'Connor et al 2014, figure 1], ie, that the observed inconsistency could have something to do with the EU/US paradox (see the last subsection, 'Confidence intervals continued'). However, as is shown in appendix B, the excess risk of PML outside America seems to disappear after around three years; already in the fourth year of natalizumab therapy, the incidence of PML in the US—notwithstanding the lower previous use of immunosuppression and the lower JCV-seroprevalence there—is practically equal to the incidence in other territories of the world (2.27‰ vs 2.35‰). The bottom line is, the EU/US paradox does not serve as an explanation, not even as a partial one, for the significantly higher occurrence of PML during months 49–72 of treatment with natalizumab in the STRATA study.

Drug holidays. Although merely loosely related to the main theme of the present article, we shall now discuss the concept of a natalizumab *drug holiday*, ie, a planned treatment interruption, because the outcomes from the STRATA trial actually demonstrate that this strategy is effective in reducing the PML incidence—thereby bringing closure to a longstanding open question. The rationale for a drug holiday is of course the obvious one: since the relative PML incidence rises sharply in the first two years on natalizumab, a break might set back the 'clock', consequently resulting in a lower risk level when therapy is resumed, which could allow even, eg, JCV-positive patients who have formerly used immunosuppressants, to take natalizumab passably safely longer than for just two years. So the idea is that these so-called *high-risk* individuals would receive natalizumab for 18–24 months, and following a pause of, say, a year, would be administered natalizumab for another 18–24 months, in the hope that the PML risk during the second on-natalizumab period is then as low as with the first time round.

No prospective studies have been undertaken so far to investigate this approach though, so that nobody really knows whether or not it works as hypothesized, as explained in a talk Mathias Mäurer gave in October 2012 [Mäurer 2012, 34:20–35:40]. Responding to a query from the audience, the speaker confirmed that this was a delicate matter, which people were somewhat racking their brains about, but that all experts currently believed that a drug holiday did not help too much and that one probably would not start counting from zero after a one-year interruption. Summarising his own words, Mäurer reiterated: 'So I cannot answer the question what happens if you take a break for one year—will you be resetted, riskwise, is it again as in the first year [of therapy], or does it simply go on ... All I can tell you is, at the moment, everyone continues counting.' Importantly, although he made that statement more than two years ago, the collective opinion about this still appears to be exactly the same [Havla et al 2013, pp 363–4].

However, the publication [O'Connor et al 2014] might change this, because the information it contains regarding the results of the STRATA study is detailed enough and in fact provides sufficient evidence to recommend a drug holiday. Concisely, patients who entered STRATA had already been given a median of 32 natalizumab infusions prior to enrolment, ie, before marketing of the drug was suspended and ongoing trials were put on hold in February 2005. All the same, the earliest case of PML in STRATA occurred in an individual who had received 33 infusions in STRATA. What is crucial, when people started natalizumab in STRATA, all of them had paused for a minimum of 57 weeks, ie, had effectively taken a mandatory one-year drug holiday. This strongly suggests that the total absence of PML from the first 30 months of treatment in STRATA is due to the year-long break everyone had had. And indeed, this is a statistically significant finding—not a single PML case in the first two and a half years of STRATA—in the sense that, if the clock was not (at least partially) set back subsequent to a natalizumab drug holiday, almost certainly PML would have emerged within STRATA ahead of the 33rd dose, as we will now see.

From [O'Connor et al 2014, figure e-3], as of late August 2013, there were 752 patients with 24 or more natalizumab infusions in STRATA. However, as just mentioned, there were no PML cases prior to the 33rd cycle [O'Connor et al 2014, p 81]. As also mentioned already, the median number of lifetime infusions at baseline in STRATA was 32 [O'Connor et al 2014, table 1]. Assume now that these 752 people had been administered only 24 infusions on average pre-STRATA (and before having to take an involuntary drug holiday), after which everybody in this cohort received another 24 infusions in the scope of the STRATA trial. If the infusion counter was not actually changed during the break these individuals were on prior to STRATA, then the p-value associated to the event of no PML cases is 0.030 (one-tailed binomial test), so that the hypothesis that a one-year drug holiday does not lower the risk of PML can be rejected. In fact, of these 752 patients, 724 had even had at least 30 infusions in STRATA, still with no-one getting PML; the corresponding p-value is 0.013.

For the sake of brevity, we shall not get bogged down in probabilities here; the interested reader is referred to appendix C, which has complete details of how the above p-values were calculated. Instead, we will now focus on

[5] A two-tailed (χ^2 or Fisher's exact) test in this situation yields a p-value greater than 0.05. It is nonetheless possible to achieve statistical significance, by modifying the null hypothesis from 'there is no difference between STRATA and post-marketing' to the weaker statement 'the incidence of PML in STRATA is not higher', which may in turn be rejected using a one-tailed test.

a few related matters. Firstly, although a drug holiday is useful in reducing one's PML risk, the question of which therapy to use as a replacement remains. Neither switching to glatiramer acetate or to one of the interferons nor *bridging* (administering intravenous methylprednisolone once per month) seems to be very effective in mitigating the recurrence of MS disease activity post-natalizumab; in contrast, fingolimod is known to be helpful in this situation [Havla et al 2013, pp 364–6]. However, the latter option, too, does not come without disadvantages: there have been voices that fingolimod should be classified as an immunosuppressive, as opposed to an immunomodulatory, agent, see [Giovannoni 2012a, Giovannoni 2015a] and also [Kornek et al 2013, p 473], so that a JCV-positive person with no previous immunosuppression receiving fingolimod while on a drug holiday might have a considerably higher PML risk when going back to natalizumab than they think. As a matter of fact, it has been suggested that teriflunomide is an immunosuppressant as well, similar to azathioprine [Gawlitza 2014, slides 4 and 27], so that on balance, perhaps the best choice in this context at the minute is dimethyl fumarate.

Of course, it needs to be determined if a drug holiday will still reset the natalizumab infusion counter when the individual concerned is on dimethyl fumarate during that period; due to the rather dissimilar mechanisms of action of natalizumab and dimethyl fumarate, this is very likely to be the case though. However, since dimethyl fumarate can actually take up to 24 weeks until it is working to the full [Kappos et al 2015, table 1], it may be worth considering starting to use it three months before stopping natalizumab, as was recently proposed [Giovannoni 2015b]. To put it differently, patients would in fact overlap natalizumab and dimethyl fumarate for the twelve weeks preceding their drug holiday.[6] That way, protection should be erected by the time multiple sclerosis flares up again, which typically happens four to seven months following the cessation of natalizumab [Havla et al 2013, p 363], occasionally even earlier [Fox et al 2014, p 1495].

Secondly, the duration of the drug holiday itself can surely be optimised. After all, it takes only approximately four months for the effects of natalizumab to vanish, ie, until important parameters such as α_4-integrin saturation and lymphocyte counts reach the levels of untreated individuals [Cree et al 2013]. Thus it is well conceivable that a shorter drug holiday, of just six to nine months, is also adequate. On the other hand, a sceptic might allege that an interruption exceeding twelve months may be in order to readjust the immune system, because with STRATA, although all subjects took a break of at least 57 weeks, the median drug holiday in fact lasted for 85 weeks with some pausing for up to three years [O'Connor et al 2014, table 1]. However, it appears improbable that this is actually necessary, given the above-cited researches into the pharmacokinetics and pharmacodynamics of natalizumab. So in all likelihood, a moderately truncated drug holiday will also do, but of course, as the changes natalizumab causes in the human body naturally show a high degree of variability among individuals, even better than settling on a recommended duration for everyone would be to personalise the length of the drug holiday. And in fact, measuring natalizumab saturation on $CD8^+$ and/or $CD4^+$ T-cells before switching medications may allow to accomplish precisely that [Wipfler et al 2014].

Thirdly, as is long-familiar, stopping natalizumab can sometimes result in a *rebound*, ie, inflammatory activity in people who quit therapy may be even higher than before treatment was initiated [Vellinga et al 2008], though the frequency of this event remains unclear, with reports ranging from zero [Clerico et al 2014, Melis et al 2014] to 22% [Sørensen et al 2014] and all the way up to 39% [Gueguen et al 2014]. Apparently, there is no consensus yet as to exactly what constitutes a rebound; moreover, distinct population subsets (all natalizumab-treated patients vs highly-active individuals only) were studied by the different investigators; a third factor possibly contributing to these seemingly disparate findings are the varying durations of the respective observation periods. Anyhow, a rebound can be severe, with some discontinuers having more than 50 contrast-enhancing lesions on MRI [Giovannoni 2013]; even fatal MS relapses have been described [Rigau et al 2012]. Finally getting to the point, until recently, people could really only speculate on the causes of this phenomenon. However, the presumptive biological explanation for some patients rebounding upon natalizumab cessation has now been discovered: *MCAM* turned out to be the magic word (literally the key to this riddle) [Schneider-Hohendorf et al 2014].

§3. Description of the problem

As outlined in the beginning, the deficiency with the way for estimating incidences as per [Bloomgren et al 2012] is that it yields figures that significantly understate the real risks. The chief reason is that, although Bloomgren and coauthors distinguish between different categories, or groups, of natalizumab-treated patients, according to the presence or absence of various risk factors, *within each category, all individuals are treated equally*. In particular, Bloomgren et al ignore the fact that some patients have received many more natalizumab infusions than others in the same category.

As an example, suppose that we wish to estimate the incidence of PML for the third and fourth years of natalizumab treatment. A patient who has so far completed 28 months of therapy visibly has had a much lower risk of getting PML *in the interval under consideration* compared to one who has already reached the 50th month (4

[6] Because health insurers may not pay for dimethyl fumarate on top of natalizumab, we include the following source [Erhardt 2014], which supplies instructions how to extremely cheaply gain access to the former.

vs 24 months). Nevertheless, Bloomgren and colleagues regard these two patients the same, ie, both are weighted equally when the risk of PML for months 25–48 is computed. Thus, the first of the two patients in this example 'dilutes' the denominator of the fraction in question, and thereby causes the PML incidence estimate to come out too low. Similarly with dropouts—if a patient decides to quit natalizumab after, say, 32 infusions, then this individual is also counted exactly like one who has received four full years of treatment. The inaccuracies resulting from this methodological error can be considerable, especially if the relative risk goes up during the respective period or when the patient numbers are small.

This has been pointed out previously, however, for instance in an article by Keith Winstein that was published in *The Wall Street Journal* [Winstein 2009]. In brief, the author questioned whether calculating the PML risk as an 'absolute percentage' (simply dividing the number of cases by the number of patients exposed) was appropriate, at all, because with chronic diseases, the long-term risk of a therapy was 'probably different than for somebody who has only a short course of the drug'. Arguing by analogy,[7] Winstein further explained that, instead of 'lumping' together everyone who had tried natalizumab, no matter how long, the actuarial method, which takes into account that risks may change over time, ought to be used in order to quantify the risk of PML associated with natalizumab. What has improved in the more than five years that have passed since the writing of the *WSJ* article? Not as much as it may seem—even though the incidence of PML is now regularly assessed for different combinations of risk factors and therapy intervals, which is progress all right, for each such group of natalizumab-treated patients, as before, it is the absolute risk that is computed instead of the actuarial risk; consequently, the PML incidence estimates are still unrealistic. We will illustrate this through the following example.

The cohort analyzed in [Bloomgren et al 2012] has a total of 4681 JCV-seropositive patients with a history of immunosuppressive treatment who had received natalizumab for at least 25 months. Of these, 52 had developed PML sometime during either the third or fourth year of therapy; hence Bloomgren et al estimated the incidence of PML in this constellation to be 52 in 4681, ie, 11.1‰ or around 1 in 90. However, less than one third of those 4681 individuals had completed four years of therapy at the time of this statistical analysis; the clear majority of patients were still within that two-year period or had already stopped natalizumab altogether. Taking this into account, using the authors' assumptions,[8] the members of this cohort had not actually received natalizumab for a combined $4681 \times 24 = 112\,344$ months, as Bloomgren et al effectively assert, but for 74 031 months only (appendix D). Therefore, the risk of PML in this setting is in reality 52 in $74\,031 \div 24$, which is 16.9‰ or approximately 1 in 59.

Note that, here, we still assume that the relative risk stays constant over the interval considered, ie, we were not using the genuine actuarial method (described later in this article), because this would have required the raw PML data, which Biogen Idec do not disclose. However, the difference between estimates derived from the actuarial method and the method just applied is all but negligible so long as the relative incidence is stable over the period at hand. We shall exemplify this in appendix B, using the first 143 cases of natalizumab-associated PML in individuals with MS (all cases reported up to the beginning of July 2011), for which the raw data are actually available [Keller-Stanislawski 2011, slide 6]; our findings are summarised in the following table:

	months 1–24		months 25–48	
method	US	ROW	US	ROW
Biogen Idec	1 : 4196	1 : 1537	1 : 493	1 : 339
actuarial	1 : 1764	1 : 876	1 : 257	1 : 187
self	1 : 2610	1 : 1109	1 : 275	1 : 190

Table 2: Post-marketing PML incidences during natalizumab therapy according to different methods of estimation.

The reader can see that the differences between the results from the method used in this article (the 'self' technique) and the actuarial one are small after the first two treatment years; for months 1–24, however, there is indeed a considerable gap, due to the relative PML incidence rising in that interval. Comparing Biogen Idec's estimates with the actuarial risks, the discrepancy is bigger still, and also persists beyond that period.

One of the nice things about having access to the raw PML data is that it also allows the visualising of how the risk changes over time (for STRATA, see appendix E):

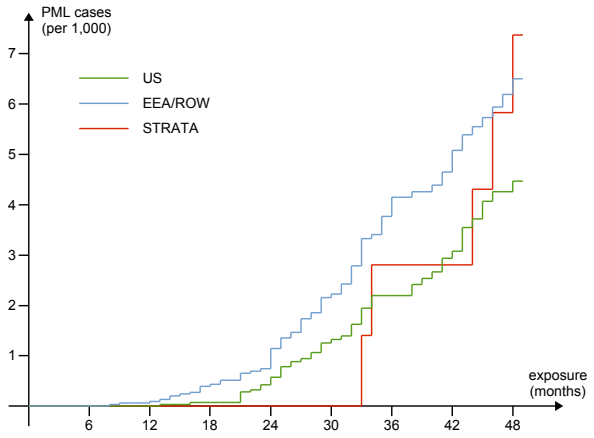

Figure 1: Kaplan-Meier curve of the (actuarial) PML incidence.

Something else that could be easily realised, with the raw

[7] 'Someone who drives a car only one day during his lifetime is less likely to be in a crash than someone who drives for 20 years.'

[8] 55% of patients JCV-positive, with 18.69% having had prior immunosuppressive treatment

data on hand, is an online risk calculator. This, too, was brought up by *Prof G*, who already in 2011 suggested 'to make a detailed PML risk calculator so that we can plug in all the variables including time on treatment and get out an individual risk' [Giovannoni 2011].

We close this section by giving a second example that demonstrates how misleading an absolute risk can be in the case of natalizumab. Using data of late August 2013, again just by considering patient numbers, a realistic estimate of the incidence of PML in STRATA between the 49th and the 72nd infusion (cf the third heuristic in the last section, p 4) was not 6 in 724 but 6 in 642 (9.3‰), whereas the corresponding post-marketing incidence was 103 in 17 934 (5.7‰) instead of 103 in 29 197 (table 14 in appendix D).[9] That is, in this instance, an accurate estimate of the total post-marketing exposure is only about 60% of what is assumed; for STRATA, however, such an estimate is nearly 90% (hence the seemingly huge difference between STRATA and post-marketing when using Biogen Idec's approach). Taking reciprocals, the resulting incidence is then 63% higher (1 : 174 vs 1 : 283) respectively 13% higher (1 : 107 vs 1 : 121), so that, with STRATA, the absolute risk estimate—1 in 121—is close to reality,[10] while in post-marketing, the true burden of therapy is much heavier than the 'absolute' method indicates. And that is because as of August 2013, 76% of patients in STRATA (484 of 641) who had received at least 49 natalizumab infusions had actually had 72 infusions, whereas in post-marketing, the same held true for only 22% (6562 of 29 197).

The following shows the occurrence of PML in JCV-seropositive STRATA participants, with or without previous immunosuppression; no correction factor is otherwise applied though (recall that the prior immunosuppressant use in STRATA was only 7%):

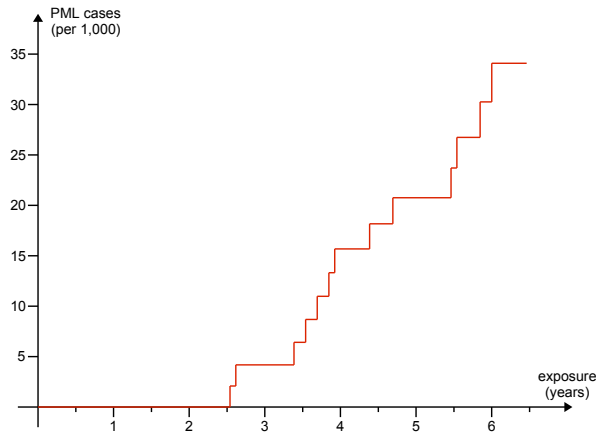

Figure 2: (Raw) incidence of PML among JCV-positive subjects in STRATA for up to six and a half years of natalizumab therapy.

[9] So even upon estimating the risks properly and despite using a correction factor, the STRATA incidence continues to be greater than what was observed in post-marketing, but by no means significantly so ($p = 0.189$ [one-tailed Fisher's exact test]).

[10] The actuarial estimate of the incidence in STRATA is 1 in 105. Perhaps the key learning outcome from these data is that in STRATA, the cumulative PML incidence with six years of natalizumab treatment (78 doses) in JCV-positive individuals was 34‰, ie, about 1 in 29.

§4. Evidence that risk-stratification is working

In the next section we will claim that estimating the PML incidences realistically also entails taking into consideration that the risk-factor algorithm has impacted patient behaviour, an assumption that was lately called into question [Cutter and Stüve 2014]. In their commentary, Gary Cutter and Olaf Stüve point out that the PML incidence estimates, somewhat surprisingly, have not come down in the past few years. For instance, as of September 2010, the chance of getting PML during the third year of natalizumab therapy (25–36 infusions) was declared to be 1.46‰ [Bozic et al 2010, figure 2 (B)]; using data as of September 2014 [TY-PAN-0597(16) 2014, slide 7], the same risk was estimated at 1.68‰ (+15.1%). The authors hence ask whether the risk-stratification algorithm has been a success, at all.

However, the real reason why the PML incidences have not only not decreased, but actually increased, is not that risk-stratification and especially JCV-serology have failed. Rather, this 'perplexing' observation, as Cutter and Stüve aptly refer to it, is again due to the systematic error in Biogen Idec's way of assessing risks described in the last section, that all individuals who have reached a particular treatment interval are weighted equally when the incidence of PML for that period is estimated. As an example, as of June 2010, there were 26 300 patients with 24+ months of exposure to natalizumab, but only 8600 with 36+ months. From this information, one can compute the corresponding *average* exposure for individuals in their third year of natalizumab, which was 7.7 months (beyond the first two years); four years afterwards, that average had climbed to 10.4 months (+35.1%), see appendix F. Ergo, without risk-stratification, the incidence of PML for this category as per Biogen Idec should have risen by a third over the 48 months from June 2010 until June 2014.

In point of fact, because the relative risk (the first derivative, mathematically speaking) is still increasing during the third year of therapy, and reaches a plateau (ie, the second derivative vanishes) only towards the end of that year,[11] the PML incidence for months 25–36 should really have gone up by 41.2%, had risk-stratification not at all worked. In detail, the (worldwide, actuarial) PML risk for the first trimester of the third year of natalizumab treatment is 0.60‰, for the second trimester the risk is 0.75‰, and for the third trimester it is 0.97‰ (table 12 in appendix B). By the end of June 2014, the experience

[11] In the US, the plateau is not actually reached until sometime during the fourth year (table 12 in appendix B).

from the third year of natalizumab therapy was almost evenly distributed: 36.99% of all patient-months fell into the first trimester, 33.14% into the second trimester, and 29.86% into the third trimester (table 18 in appendix F). Four years before, however, there was a very significant bias in that the first trimester carried more than twice the weight of the third trimester (46.47% vs 21.45%). Thus, making allowance for the different weight distributions, in addition to the 35.1% from above, one would expect the risk of PML, estimated in the manner of Biogen Idec, for this period to have grown by another factor of 1.045,[12] so that, in total, the PML incidence during the third year of natalizumab therapy should have increased by approximately 41.2% ($1.351 \times 1.045 = 1.412$) since mid-2010. Even so, as noted in the first paragraph of this section, the observed increase was only 15.1%, which in all likelihood is due to the expected increase being 'cushioned off' by risk-stratification, ie, there are two effects at work here, namely the flaw in Biogen Idec's method and PML risk-stratification, and the latter partially cancels out the former.

If the reader is not yet convinced of the fruitful implementation of risk-stratification, there is another approach to see that it must have had some impact. In 2012, there were 122 cases of natalizumab-associated PML; during the same period, the total natalizumab exposure rose by 66 490 patient-years, so that there was one case per 536 patient-years. (References for all figures used in this paragraph may be found in appendix G.) In contrast, the PML incidence between 4th September 2013 and 2nd September 2014 was just 1 per 720 patient-years, as there were 94 PML cases on 67 649 additional patient-years for that period.[13] So over the course of 20 months, there was a 24.3% reduction in the relative number of cases, which is statistically significant ($p = 0.0246$ [one-tailed Fisher's exact test]).

In §5, we shall continue to work with the cohort from [Bloomgren et al 2012], which includes PML cases only through the end of February 2012. Hence we also need to show that risk-stratification was already functioning prior to this date. Consider therefore again the PML incidence in the third year of natalizumab treatment. As of 31st March 2011—about the time JCV-serology testing became generally available—the total post-marketing experience with months 25–36 of therapy comprised 26 536 years of exposure and 65 PML cases, which works out to one case per 408 patient-years. Eleven months later, at the data cut-off for the study by Bloomgren et al, there were a further 23 PML cases in said category with 11 324 patient-years more, ie, one case per 492 patient-years (all details are supplied in appendix G). Thus, already in the first year of routine JCV testing, the PML incidence during months 25–36 of natalizumab treatment was 17.1% lower than earlier. In fact, taking the weights of the trimesters into account, exactly as we did with our first example in this section, the additional 11 324 patient-years correspond to 11 728 in pre-April 2011 terms, so that the relative frequency was really one PML case for every 510 'regularised' patient-years (−20.0%).

Another way to see that deriskification must already have been taking place before March 2012 is to examine the relative number of PML patients with prior immunosuppressive treatment. As of 4th March 2011, 39 out of 93 (42%) individuals diagnosed with PML for whom this information was available had used immunosuppressants prior to natalizumab; one year later, as of 29th February 2012, 68 out of 197 (35%) had [TY-PAN-0597(16) 2014, slides 26–27]. This means that with the $197 - 93 = 104$ PML cases that occurred between 4th March 2011 and the end of February of the following year, of the affected people only $68 - 39 = 29$ (28%) had formerly been administered immunosuppressive medication, which is a significantly lower proportion than the 39 of 93 from before ($p = 0.0273$ [one-tailed Fisher's exact test]).

In summary, although the risk-factor algorithm has by now definitely kept several dozen patients from developing PML, the reason why the reported incidences are still higher than in 2010 lies in the way in which they are calculated. Looked at it like this, the observation made by Cutter and Stüve actually represents a fourth heuristic argument (see §2) as to why something is not right with Biogen Idec's method of assessing risks.

A related matter is, even though risk-stratification has been useful in reducing the PML incidence, the price paid is a high one, since the 26 cases 'saved' in the 12-month period from September 2013 compared to the 2012 calendar year imply that somewhere between 3000 and 5000 patients must have been taken off natalizumab, in spite of the fact that over 95% of them could have safely continued using the drug. Of course, most of these deriskified individuals were presumably put onto some other therapy. However, the consensus seems to be that, with the possible exception of alemtuzumab, none of the agents currently approved for the treatment of relapsing MS is as effective as is natalizumab, so probably the majority of patients fared a lot better ahead of being switched. Given that, courtesy of the CD62L bloodtest and the check for lipid-specific IgM bands in cerebrospinal fluid, natalizumab needs to be stopped in only approximately 250 people in order to prevent 26 cases of PML, the present handling of this problem is certainly far from optimum. Which is highly regrettable, not least because the situation not just affects patients and their families, but also their treating physicians—in Gavin Giovannoni's words [Giovannoni 2014c]: 'I still have sleepless nights over natalizumab and PML. Despite advising all our high-risk patients to come off the drug a few patients want to stay on the drug. These are typically patients who have had very bad MS and are now doing very well on natalizumab.

[12] $\frac{36.99\% \times 0.60‰ + 33.14\% \times 0.75‰ + 29.86\% \times 0.97‰}{46.47\% \times 0.60‰ + 32.07\% \times 0.75‰ + 21.45\% \times 0.97‰} = 1.045$

[13] Strictly speaking, for the period from 1st October 2013 until 30th September 2014.

I keep telling the MS Team that it is simply a numbers game and it is only a matter of time before we have our first case of PML at the Royal London Hospital. I fear that day!'

§5. Improving risk estimates further through more realistic assumptions

The other reason why some of the PML incidence estimates are falsely low is that the assumptions Bloomgren et al make are not realistic, in two ways. Firstly, too high a proportion of the previous use of immunosuppressants is assumed. Secondly, they suppose that this percentage, as well as the percentage of JCV-positive patients, is independent of natalizumab exposure, ie, they do not take account of the reality (§4) that individuals who are at a high risk of PML disproportionately often cease natalizumab therapy, which, however, is really the whole point of dividing patients into categories of different risks.

The relevance of the latter was again emphasised by Gavin Giovannoni on his blog. In a comment answering a question of one reader, Giovannoni remarked that the subgroup of natalizumab-treated patients having had 30 or more infusions was likely 'enriched' with patients at a lower PML risk, due to high-risk individuals coming off the drug [Giovannoni 2012b]. Importantly, he posted this in March 2012, ie, about the time Bloomgren et al were conducting their analysis. Similarly, when discussing possible causes of the estimated PML incidence in STRATA being higher, the lead investigators from this trial state that the natalizumab post-marketing population was 'dynamic' and might have changed 'in response to expanding knowledge of PML risk factors' [O'Connor et al 2014, p 85]. As far as the former assumption is concerned, the authors themselves openly acknowledge this as a limitation [Bloomgren et al 2012, p 1878]: 'Recent data from the Tysabri Observational Program (TOP) ... indicate that the percentage of patients with prior use of immunosuppressants is approximately 15%, a percentage slightly lower than, but relatively consistent with, data from the TYGRIS study (20.3%).' Actually, this very last affirmation ('relatively consistent with') is inaccurate, since the two respective ratios in fact differ extremely significantly ($p < 0.0001$ [χ^2 test with Yates's correction]). We shall begin by addressing the question of how to obtain a realistic estimate of the frequency of the past usage of immunosuppressive medication; we will then deal with the issue of patients quitting natalizumab for fear of PML.

Prior IS use. From [Bloomgren et al 2012, p 1873], in order to estimate the proportion of natalizumab-treated patients with prior immunosuppressive therapy, the findings from TYGRIS are extrapolated. That is, Bloomgren et al assume that 14% of patients in America and 23.5% elsewhere are positive for a history of therapy with an immunosuppressant. Observe that the figure 20.3% quoted in the last paragraph is not just the average of 14% and 23.5%, and presumably comes from the fact that of the 6467 TYGRIS enrollees, 2203 were in the US and 4261 in the EEA/ROW [Kappos et al 2011a, p 746].[14] To the authors' credit, they do not actually suppose that 20.3% of natalizumab-treated individuals have had immunosuppression; rather, they calculate 'the weighted proportion of prior use of immunosuppressants in the TYGRIS-U.S. study and in the TYGRIS-Rest of World study'. For their cohort, this means that 18.34% (18 261 of 99 571) of all patients vs 18.69% (8509 of 45 533) of those longer than two years on natalizumab are assumed to have undergone immunosuppressive pretherapy.[15]

As mentioned already, Bloomgren et al concede that the TOP trial suggests that the percentage of prior exposure to immunosuppression is lower than what they assume for their analysis. There are several remarks to be made. First of all, TOP, although a multinational study, does not include US patients, ie, the 15.3% (697 of 4541) previous use of immunosuppressants in patients enrolled as of 1st December 2012 [Butzkueven et al 2014, table 1] reflect experience in the EEA/ROW only (and are significantly different from the 23.5% seen in TYGRIS-ROW, $p < 0.0001$ [χ^2 test with Yates's correction]). Moreover, there is one country in TOP, namely the Czech Republic, that acts somewhat as an outlier, in the sense that it unduly increases the proportion of prior immunosuppression in TOP—as of 1st June 2011, almost half (172 of 349) of Czech people in TOP had formerly been treated with immunosuppressants [Kappos et al 2011b, figure 1]; excluding these 349 individuals decreases the frequency of past immunosuppressive therapy in TOP to 12.5%. Finally, recruitment with this clinical trial was not actually proportional to the clinical use of natalizumab in the EEA/ROW, so extrapolation has to be performed cautiously. For example, as of 1st December 2012, 13.4% of subjects in TOP were based in the Czech Republic, while merely 3.9%, 6.5% and 2.1% were from France, Italy respectively Spain [Butzkueven et al 2014, table 2], so that more patients in the Czech Republic were enrolled than patients from three of the six 'principal markets' of Tysabri [Biogen Idec 2013, p 10] combined. An alternative way to infer that enrolment in TOP must be skewed is to consider the number of PML cases separately for each participating country—among the first 143 cases of natalizumab-associated PML in patients with MS (all cases up to July 2011), there were twelve people from France, six each from Italy and Spain, but only a single one from the Czech Republic [Keller-Stanislawski 2011, slide 6].

This last way of looking at it suggests how to perhaps best estimate the frequency of previous use of immunosuppressants with natalizumab-treated individuals in the

[14] $(2203 \times 14\% + 4264 \times 23.5\%) \div 6467 = 20.3\%$

[15] Apparently, 54.3% of all patients were in the US, and 50.6% of those on the drug for 25+ months (follows from solving the equation $p \times 14\% + (1 - p) \times 23.5\% = 18.34\%$ resp. 18.69% for p).

EEA/ROW, given that the exact geographic distribution of patients is unavailable: by also computing a weighted proportion, where, however, the weight of a country is not determined by its enrolment in TOP but by the number of PML cases it has seen. This will assign more weight to territories having a comparatively high prior usage of immunosuppressants, so that the figure obtained in this fashion will still be a slight overestimate, ie, the precise frequency of the past use of immunosuppressive agents in the EEA/ROW will be marginally lower.

We carry this out in appendix H using the PML data from [Keller-Stanislawski 2011] and where we further ensure not to produce an underestimate (and consequently overstate the risk of developing PML) as follows. With countries not involved in TOP, a quarter prior use of immunosuppressants is assumed; for countries that are in fact participants in TOP, we do not directly use the frequency from this study, but rather the smallest proportion that, with probability exceeding 95%, is not smaller than the real proportion.[16] Hence, Switzerland, which is not included in TOP, is assumed to have 25% previous use of immunosuppressants, whereas for the United Kingdom, a proportion of 7.1% is used—as of 1st June 2011, there were 87 patients from the UK enrolled in TOP, of whom two (2.3%) had at one time received immunosuppression [Kappos et al 2011b, figure 1]; if there was 7.0% former use of immunosuppressive therapy in the UK, then, in a random sample of 87 people, the odds of two (or fewer) having had treatment of this type would be greater than 5% and therefore this event would be statistically insignificant; on our assumption of 7.1% earlier exposure to immunosuppressants, the chances of this happening are actually less than 5%.

The resulting estimate is that the frequency of prior use of immunosuppressants in natalizumab-treated patients in the EEA/ROW is 17.7%. Coincidentally, pooling the cohorts from TYGRIS-ROW and TOP—again excluding the 349 individuals from the Czech Republic enrolled as of 1st June 2011, for the above reason—yields a proportion of 18.1% previous exposure to immunosuppressive drugs.[17] Given these virtually identical estimates arrived at very differently, we shall henceforth assume that the sought unknown is 18%. Recalculating the risk of PML for months 25–48 of natalizumab treatment in JCV-positive patients with immunosuppressive pretherapy, as in the last section, but assuming 18% prior use of high-risk agents in the EEA/ROW instead of 23.5% then gives an incidence of 52 in $63\,281 \div 24$,[18] ie, 19.7‰ or approximately 1 in 51 (see also table 13 in appendix D).

[16] In effect, for every country in TOP, we compute a 90% confidence interval and then use its upper endpoint as our estimate of the frequency of the prior administration of immunosuppressive medication in that country.

[17] $[4264 \times 23.5\% + (697 - 172)] \div [4264 + (4541 - 349)] = 18.1\%$

[18] $74\,031 \times (50.6\% \times 14\% + 49.4\% \times 18\%) \div 18.69\% = 63\,281$

Original vs second-generation JCV serology testing. In contrast to the previous subsection, one other assumption that Bloomgren et al make, namely regarding the proportion of JCV-positive individuals (55%) among all patients with MS, is—by today's standards—too conservative, leading to a slight overestimate of the PML incidences. However, at the time of their work, this assumption was fully justified, since as of early 2012, it was still the original JCV antibody ELISA that was in use, and with this assay, in joining four different populations comprising a total of 5896 subjects, the authors found that about 54.9% of natalizumab-treated patients are positive [Bloomgren et al 2012, table 2]. This figure was subsequently confirmed: in STRATIFY-2 (a study conducted in MS patients in America only), at baseline, 54.7% of the 19 537 participants tested positive with respect to JCV-antibodies in serum [Bozic et al 2012], whereas in JEMS (a multinational cohort, but not involving the US), 57.1% of 7724 did [Bozic et al 2014].

As just indicated, what somewhat changed the picture was the replacement of the original two-step ELISA with the second-generation assay (STRATIFY JCV DxSelect) [Lee et al 2013] for regular clinical testing at the beginning of April 2012 [Outteryck et al 2014, p 823], because this improved assay produces a positive diagnosis a little more frequently than does its predecessor. The diagnostic agreement between the two methodologies is described in [Lee et al 2013, table 4]; the respective PPA and NPA make it possible to retrospectively estimate the JCV-seroprevalence in people with MS for the second-generation ELISA (ie, as if this assay had already been available at the time of the above-named trials). All relevant calculations have been delegated to appendix I, but the result is that the 54.7% JCV-positivity from STRATIFY-2 and the 57.1% measured in JEMS with the original ELISA correspond to 57.3% and 59.4%, respectively, with the second-generation assay. Therefore we will henceforward suppose that the JCV-seroprevalence is

$$(57.3\% + 59.4\%) \div 2 = 58.4\%, \tag{2}$$

which is also justified since among those treated with natalizumab for longer than two years, more or less exactly half of them were based in the US at the time of the analysis by Bloomgren et al (see footnote 15 on the previous page). Thus, recalculating the risk of PML in JCV-positive individuals with previous exposure to immunosuppression for months 25–48 of natalizumab therapy but assuming that 58.4% of all patients instead of 55% are positive for JCV antibodies in serum then gives an incidence estimate of 52 in $67\,280 \div 24$,[19] ie, 18.5‰ or about 1 in 54 (see again table 13 in appendix D).

High-risk dropouts. We now turn our attention to what is the trickiest part of getting the assumptions right: tak-

[19] $63\,363 \times 58.4\% \div 55\% = 67\,280$

ing care of the fact that individuals whose JCV status is positive often quit natalizumab in order to avoid developing PML. In a way, we need to incorporate the consequences of the risk factor algorithm into itself. Not surprisingly, here, too, there are no hard data available, so once more we have to extrapolate from clinical studies, and again we need to be very cautious. Therefore, so as to obtain a fair estimate of patients' behaviour, we will average the outcomes of four independent observational studies, from four different countries, and demonstrate that the predictions thus derived are very much consistent with the overall discontinuance rates for each year of treatment in the routine (commercial) use of natalizumab. With all four studies, the goal was to investigate the dropout rate in patients receiving natalizumab, either according to, or in case of positive, JCV status.

The first study, performed in Spain, included 104 individuals [Tur et al 2012], of whom 48 were JCV-positive and had been treated with natalizumab for at least two years. For this subgroup, the rate of discontinuation was 60% (6 of 10) in patients with, vs 23.7% (9 of 38) in patients without, prior immunosuppression. In contrast, all JCV-positive patients with less than 24 months of natalizumab exposure—irrespective of pretherapies—as well as everybody negative for JCV antibodies opted to keep their treatment strategies (ie, stayed on natalizumab).

The second study, carried out in France, has the largest cohort [Ongagna et al 2013]; in all, 292 individuals were followed. Of the 150 participants who were JCV-positive, 38 decided to quit natalizumab, with 15 having received immunosuppressive pretreatment. As it seems, the total number of JCV-positive patients with past immunosuppressive therapy was not recorded for this trial. However, given our estimate of the prior immunosuppressant usage in France obtained from TOP (13.8%, appendix H), we will assume that 21 of these 150 persons had previously received one of the high-risk agents. Hence the cessation rate was 71.4% (15 of 21) and 17.8% (23 of 129) in patients with respectively without earlier immunosuppressive therapy. Note that both these estimates concern all natalizumab-treated patients, regardless of exposure, so that the discontinuation rates in the period that we are interested in (months 25–48) should actually be higher.

In the third study [Lonergan et al 2013], from Ireland, the dropout rate was 38.2% (21 of 55) in JCV-positive and 12.3% (7 of 57) in JCV-negative patients. Of note, there was no significant difference with regard to treatment durations or the proportions of the prior immunosuppressant use among JCV-positive patients who ended natalizumab therapy vs those who continued. However, there was one patient who stopped treatment not alone on the grounds of being infected with JCV but also because of having been exposed to an immunosuppressive drug in the past. Excluding said individual yields a dropout rate of 37.0% (20 of 54) with JCV-positive patients never having taken immunosuppressive medication.

The fourth, and most recent, study was conducted in Kuwait [Alroughani 2014]. Here, the investigator found that the discontinuation rate among JCV-positive natalizumab-treated patients was 59.4% (19 of 32).[20] As with the French and the Irish trials, the number of individuals formerly exposed to immunosuppression is not provided. However, even if eight patients (25%) had in fact undergone such treatment and all but one of them quit natalizumab, the cessation rate in patients without immunosuppressive pretherapy was nonetheless 50% (12 of 24). Furthermore, natalizumab exposure did again not seem to significantly influence individuals' decisions. We summarise the results from this meta-analysis in the following table:

	dropout rate in JCV+ pts.		
country	without prior IS therapy	with prior IS therapy	dropout rate in JCV− pts.
Spain	23.7%	60.0%	0%
France	17.8%	71.4%	
Ireland	37.0%		12.3%
Kuwait	$\geq$50%		

Table 3: Discontinuation rates for months 25–48 of natalizumab treatment as per different studies.

Recall now that the findings from the French and the Kuwaiti studies rely on additional assumptions, namely, regarding the frequency of the earlier use of immunosuppressants. Moreover, as indicated by the '$\geq$' symbol, the figure obtained from the Alroughani trial is not an exact fraction, but a lower bound. Thus, to estimate the cessation rate for months 25–48 of natalizumab treatment in JCV-positive patients with no immunosuppression, we do not simply take the ordinary average (arithmetic mean) of the four percentages in the second column of table 3 but their median, ie, our cumulative dropout rate for the third and fourth years of natalizumab administration in JCV-positive individuals without previous exposure to an immunosuppressant is $(23.7\% + 37\%) \div 2 = 30.4\%$.

In order to model the conduct of JCV-positive natalizumab-treated patients with former immunosuppressive therapy, as well as that of JCV-negative patients, we can avail ourselves of just two trials, so we shall proceed in a different manner: with either constellation, what we will do is use only the less extreme of the two proportions in question, to make sure that our resulting estimate of the

[20] This trial was reported as a response to a similar one from the Netherlands [van Rossum et al 2014a], where just 3% (2 of 75) of JCV+ patients quit natalizumab and which astonished Alroughani. Certainly generalization is difficult—prior to 2013, the annual cessation rates hovered around 14%; if only 3% of JCV+ individuals stopped each year, then the dropout rate in JCV− patients would necessarily have been almost ten times as high. Not very plausible, which is also why we do not consider the Dutch study when estimating patient behaviour. (NB: A much higher discontinuation rate was later seen in that same cohort [van Rossum et al 2014b].)

incidence of PML is not an overestimate. For JCV-negative patients, this means that we will assume a 12.3% cessation rate during months 25–48 (the greater of the two values in the fourth column of table 3); likewise, for JCV-positive individuals with prior exposure to immunosuppression, for the same treatment interval, we will suppose a cessation rate of 60% (the lesser estimate in the third column of table 3).

The dropout rates in natalizumab-treated patients established above—60%, 30.4%, 12.3%—concern a two-year interval; however, what we will be ultimately interested in are the respective annual discontinuation rates, which we therefore provide in our next table. These percentages are calculated under the hypothesis that they are the same for both years,[21] which should be a reasonable assumption to make at least for the twelve months after JCV serology testing became generally available (ie, from about March 2011 until February 2012):

risk constellation	annual dropout rate during months 25–48
JCV+, prior IS	36.8%
JCV+, no prior IS	16.6%
JCV−	6.4%

Table 4: Annual discontinuation rates for each of the third and fourth years of natalizumab treatment according to JCV serostatus and prior or no prior immunosuppressive therapy.

As JCV-positive patients, in particular those with prior immunosuppression, are much more likely to quit therapy, the proportion of JCV-positive natalizumab-treated patients goes down with time, as does the rate of prior immunosuppression. Using the percentages from the last table, one can easily estimate these proportions:

natalizumab exposure	JCV+	prior IS
24 months	58.4%	16.0%
36 months	54.6%	12.6%
48 months	50.9%	9.9%

Table 5: Estimated long-term frequencies of PML risk-factors.

So even on the assumption of absolutely no deriskification during the first 24 months, already after four years of natalizumab treatment, in the long-term, only about half of patients are JCV-positive anymore, and of those, just one in ten has formerly been treated with immunosuppressants.

Since we suppose that, as of March 2011, 55% of all natalizumab-treated patients were JCV-seropositive, with 16% having had immunosuppressive pretreatment (14% in the US, 18% in the EEA/ROW), as discussed in the previous subsections, the resulting estimated overall stop rate for each of the third and fourth years of natalizumab treatment as it was during the period from March 2011 until February 2012 is therefore

$$\begin{aligned} 55\% \times 16\% \times 36.8\% \;+\; 55\% \times 84\% \times 16.6\% \\ +\, 45\% \times 6.4\% \;=\; 13.8\%. \end{aligned} \tag{3}$$

Note that we are deliberately working on the assumption of 55% JCV-seroprevalence, not 58.4%, because for the above period, it was still the original JCV antibody ELISA that was being employed, see p 11. For completeness, with the 58.4% positivity of the second-generation JCV assay, the dropout rate (3) increases to 14.2%.

Earlier we said that our estimates were going to be very much in line with the observed overall dropout rates in the commercial use (clinical practice) of natalizumab. In order to be able to verify this claim, we first need to compute these rates. We will do this for each year of therapy, as well as for different periods, the latter because it is interesting to see how the discontinuation rates have evolved over time. In fact, we will not carry out all calculations here—instead, we shall give two examples; the remaining cases are analogous.

In our first example, we will compute the cessation rate for the third year of natalizumab therapy prior to April 2011 (ie, roughly up to the point where JCV testing became widely available). As of 31st March 2010, in total, there were 21 300 individuals who had received natalizumab in the post-marketing setting for at least 24 months [Biogen Idec 2010, slide 18]. One year later, 18 700 patients had been treated for at least 36 months [Bozic 2011, slide 5]. Accordingly, the dropout rate for months 25–36 of natalizumab therapy up to and including the first quarter of 2011 was

$$(21\,300 - 18\,700) \div 21\,300 = 12.2\%.$$

Our second example is a little less trivial; we will show how to work out the discontinuation rate for the fourth year of natalizumab therapy between 1st April 2013 and 31st March 2014. On 31st March 2012, 30 600 patients had had 36 (or more) months of natalizumab exposure. A year later, there were 41 100 such individuals, so that 10 500 patients must have begun their fourth year of natalizumab treatment sometime between 1st April 2012 and 31st March 2013. Of these, 8 700 actually finished the fourth year within the following twelve months.[22] So the dropout rate during the fourth year of therapy for the 12-month period starting on 1st April 2013 was

$$(10\,500 - 8\,700) \div 10\,500 = 17.1\%.$$

[21] Example. 60% of JCV+ patients with previous IS therapy quit during months 25–48; 40% are still on natalizumab at the end of that two-year interval. So $\sqrt{0.4}$ continue to take the drug at the 'halfway point', ie, the annual dropout rate is $1 - \sqrt{0.4} = 0.368$.

[22] As of 31st March 2013, there were 26 600 patients with at least 48 months of therapy; a year later, 35 300 were in this category.

We refer to appendix J for a more detailed justification, should the reader not be fully convinced that this reasoning is correct. Continuing in this fashion, we obtain the following results (the raw patient numbers used as a basis are also given in appendix J):

	dropout rate during months			
	1–12	13–24	25–36	37–48
up to 03/2011	18.6%	13.7%	12.2%	
04/2011–03/2012	12.8%	13.5%	15.6%	
04/2012–03/2013	12.3%	15.4%	13.9%	
04/2013–03/2014	18.4%	19.6%	16.5%	17.1%

Table 6: Development of the overall annual discontinuation rates over time in the clinical use of natalizumab.

Although somewhat off-topic, we will now make a few short remarks regarding the preceding table. First of all, up to March 2011 and again from April 2013, the dropout rate was relatively high during the first year of treatment, possibly reflecting the fact that natalizumab users quit therapy not for fear of PML, but for lack of efficacy, eg, due to the development of neutralising natalizumab antibodies.[23] Secondly, the percentage of dropouts for the third year of treatment was actually a bit higher between April 2011 and March 2012 than during the next twelve months (15.6% vs 13.9%), perhaps because patients were deferring the decision whether or not to continue until after obtaining their JCV status. (So that fewer people than expected stopped natalizumab in the months prior to JCV testing becoming accessible, leading to a slight 'overshoot' in the first year after its introduction.) Thus the 13.9% cessation rate during months 25–36 observed between April 2012 and March 2013 may be considered the 'steady-state' rate for this interval of therapy, cf our estimate (3). Thirdly, with all treatment periods except the first year, dropout rates peaked after March 2013, presumably since that was when risk-stratification *really* started to kick in (see also §4).

After this digression, we finally continue, and at long last finish, deriving our estimate of the risk of PML for months 25–48 of natalizumab therapy in JCV-positive patients with prior immunosuppression. To this end, recall that one finding of our meta-analysis (see the discussion just before table 4) is that 60% of JCV-positive patients with former immunosuppression quit natalizumab sometime during months 25–48 of therapy. Since JCV serology testing did not become generally available until the first quarter of 2011, however, when estimating the proportion of people in the cohort from [Bloomgren et al 2012] having the above risk factors, one cannot simply use the percentages from table 5 as those are long-term frequencies (ie, computed as if JCV testing had been performed regularly right from the market introduction of natalizumab). Hence we shall proceed as follows. For all infusions administered up to and including March 2011, we will suppose that there was no deriskification at all (ie, no correlation between dropout rate and PML risk factors), so that throughout, 58.4% of all patients are assumed to be JCV-seropositive with 16% having had immunosuppressive pretherapy. For infusions given after March 2011, we will apply the monthly discontinuation rates that correspond to the annual rates from table 4. As an example, for a JCV-positive individual who has previously used immunosuppressants, the monthly chance of dropping out after two years of treatment is therefore

$$p := 1 - \sqrt[12]{1 - 36.8\%} = 1 - \sqrt[12]{0.632} = 3.75\%.$$

Using the data from [Bloomgren et al 2012, figure 1] and [Bozic 2011, slide 5], approximately 11 000 patients received their i-th natalizumab infusion during the 11-month period from April 2011 through February 2012, for each $i = 25, 26, \ldots, 48$. (The precise numbers vary with i and can be found in appendix K.) For a fixed value of i, $35 \leq i \leq 48$, assuming a uniform growth rate, this means that around 1000 patients received the i-th infusion in April 2011, another 1000 in May 2011, ..., and the remaining 1000 in February 2012. Those who had their i-th dose in April 2011 spent exactly one month in the period of available JCV testing, those who had it in May two months, and so on. Thus, if $q := 1 - p$ denotes the monthly probability of a JCV-positive individual with prior immunosuppression continuing therapy, then such a patient originally in the first group had probability q of in fact receiving infusion no. i, a patient in the second group had probability q^2, ..., while one in the last group had probability q^{11}. By replacing q with the appropriate value, one can of course do the same for JCV-positive patients with no immunosuppressive pretreatment, as well as for JCV-negative people. In combining the results obtained, one may then deduce the proportion of JCV-positive individuals with prior immunosuppression among all patients who received the i-th natalizumab infusion (in the post-marketing setting) between April 2011 and February 2012; with this percentage on hand, patient numbers may then be interpolated in the usual fashion.

Actually, for $25 \leq i \leq 35$, one more aspect needs to be taken into account, namely, that (on our assumptions anyway) there was no deriskification during the first two years of natalizumab therapy, see table 5. For example, in keeping the notation from the last paragraph, the chance of a JCV-positive individual with prior immunosuppression who had had 14 natalizumab infusions as of March 2011 to in fact receive (in February 2012) their 25th infusion was not q^{11}, but q. Although that person did receive eleven infusions after JCV testing became available, ten of these fell into the interval of no risk-stratification, ie, the first 24 months of treatment (where natalizumab is deemed sufficiently safe regardless of an individual's risk

[23] It is not at once clear why this figure was considerably lower from April 2011 to March 2013.

factors). By the same token, the chance of a patient to receive their 30th infusion in December 2011 was not q^9, but q^6—even though nine infusions were given after JCV testing was made available, only six of them occurred in the period where deriskification actually takes place.

We carry all this out in appendix K, giving some more explanations there as well. The result is that the total exposure among JCV-seropositive natalizumab-treated patients with prior immunosuppressive therapy in the cohort from [Bloomgren et al 2012] equals 64 147 months, so that the corresponding estimate of the incidence of PML in this category is 52 in 64 147 ÷ 24, which is 19.5‰ or about 1 in 51.

We emphasise once more that this estimate was calculated assuming that the relative risk is constant throughout the interval in question. This assumption is clearly false when all JCV-positive patients are considered; recall that the risk of PML reaches a plateau only after about three years (see table 12), ie, is still increasing during months 25–36 of natalizumab treatment. However, it is unknown if this is also the case for individuals with prior immunosuppression—perhaps the risk levels off earlier in this group? Should the plateau not already be reached after two years, computing the above risk using the true actuarial method (which requires the raw PML data, see §9) would indeed result in an incidence estimate that is somewhat higher still.

§6. Estimating the risk of PML in JCV-seropositive patients without prior immunosuppression

In this section we will estimate the incidence of PML in JCV-positive patients not previously treated with an immunosuppressive agent. Somewhat oddly, we shall first calculate the risk for months 49–72 of therapy and thereafter that for months 25–48, the reason being that with the former period, we may safely assume that the relative risk does not rise any further (ie, stays constant), which, as noted before, does not actually apply to the latter interval; the risk of PML on natalizumab reaches a plateau only around the beginning of the fourth treatment year (table 12 in appendix B). Once we have computed the PML incidence for the fifth and sixth years of therapy, we will in fact use our result to also derive a reliable estimate of the risk with the third and fourth years.

Months 49–72. For months 49–72 of natalizumab therapy, according to Biogen Idec's latest quarterly update, the PML risk is 6.1‰ [TY-PAN-0597(17) 2014, slide 9], estimated using data from the post-marketing setting as of 5th March 2013 (at which time there were 343 PML cases). In order to correct the error resulting from Biogen Idec's way of computing risks, it is not enough to know how many individuals have received natalizumab for at least 49 months, as explained in §3; rather, one also has to know what fraction of patients in this group have been treated with natalizumab for 50, 51, . . . , 72 months. At first, the sole useful piece of data that seems to be available is that by the end of March 2013, 26 600 people had received natalizumab (in the post-marketing setting) for at least 48 months [TY-PAN-0597(2) 2013, slide 9], but fortunately, the paper [Bloomgren et al 2012] has more details: from table 1, by the end of February 2012, there were 29 085 people with 37 or more months of natalizumab therapy, of whom 14 239 had even received the drug for 49 months (3 596 for 61 months). However, what we really need to know is how many individuals had had 49, 61 respectively 73 months of treatment as of early March 2013 (ie, one year later).

Actually, since the incidence is by definition a proportional measure (number of cases per 1000), we only need to know the *relative* frequencies, ie, the percentage of individuals among all those with at least 49 months of natalizumab treatment who have in fact been administered this medication for 61 (respectively 73) months. Anyone with 49 therapy months as of March 2013 must obviously have had 37 months one year earlier; similarly, someone with 61 (73) months had necessarily had 49 (61) months a year before. Not everybody in the above category of 29 085 natalizumab users with more than 48 months' exposure was still on the drug a year later, of course, but if we assume that the discontinuation rate is uniform, then by what we noted in the preceding paragraph, we already have all the information that we require.[24] The details are delegated to appendix L; the result is that as of the beginning of March 2013, compared to what Biogen Idec assume, just over half (53.2%) as many infusions had in fact been given during months 49–72 of therapy, and so the corresponding PML incidence is

$$\frac{1}{0.532} = 1.88 \tag{4}$$

times what is reported, ie, 11.5‰ (vs 6.1‰). As in §5, one further needs to take into account that high-risk patients quit natalizumab therapy disproportionately often. However, unlike when we estimated the risk in JCV-positive individuals with former immunosuppressive therapy for months 25–48 (where over 60% of infusions had been given before JCV serology testing became available), in the present situation it is actually justified to assume the

[24] As was pointed out in August 2012 [Cervera 2012] in a comment on [Bloomgren et al 2012], the risk of natalizumab-associated PML seems to increase 'abruptly' when treatment is continued for 61–72 months. Therefore it is conceivable that a patient with five years of natalizumab therapy as of, eg, July 2012, was somewhat more likely to quit in the following months than somebody with just four years; consequently, the dropout rate in question may not have been entirely uniform over the period under consideration, with individuals longer on treatment potentially stopping a little more frequently. Hence the estimate of the incidence of PML for months 49–72 we will work out may be a slight underestimate of the reality, but the difference should almost certainly be small.

long-term proportions from table 5. For one thing, this time we are working with data of March 2013, ie, when JCV testing had already been generally available for almost two years, so that most natalizumab users, certainly those with more than 48 months of exposure, were aware of their JCV status (by the end of March 2013, in the EEA/ROW alone, more than 80 000 samples had been tested for the presence of JCV antibodies [Unilabs 2013]). For another, nearly all PML cases in the fifth and sixth years occurred after JCV testing had been introduced—as of early July 2011, merely seven cases of natalizumab-associated PML had occurred after more than four years of treatment [Keller-Stanislawski 2011, slide 6], while as of 5th March 2013, there must have been 62 to 66 such cases (see appendix L). Thus, we shall not assume that 58.4% of patients in the subcohort in question are JCV-positive, but 50.9%, and that only 9.9% instead of 18% have had previous immunosuppression (so that 90.1% instead of 82% did not have prior exposure to immunosuppressants), which are the long-term frequencies for individuals having had four years of natalizumab treatment, see table 5. Since a few of the PML cases during months 49–72 actually did occur in individuals who had not yet been able to find out their JCV status, this last assumption is not completely accurate and leads to a risk estimate that is slightly higher than the true PML incidence. On the other hand, as mentioned already, we also suppose that no further deriskification took place (after 48 months of therapy), which is also not quite realistic, and means that we will in fact underestimate the PML incidence. However, these two deviations should cancel out each other by and large, and surely neither makes a difference that is anywhere near of being significant.

Putting together everything we just noted, the at-risk population in this constellation is actually greater than Biogen Idec assume—$50.9\% \times 90.1\% = 45.9\%$ compared to $55\% \times 79.7\% = 43.8\%$—and hence the 11.5‰ PML incidence (4) needs to be divided by

$$\frac{50.9\% \times 90.1\%}{55\% \times 79.7\%} = 1.046,$$

so that the definite estimate of the PML risk in JCV-positive patients without previous immunosuppressive treatment for months 49–72 of natalizumab therapy is equal to 11.0‰ (about 1 in 91).

Months 25–48. To calculate a proper estimate of the risk of PML for months 25–48 of natalizumab therapy in JCV-positive patients without prior immunosuppression, as hinted at the beginning of this section, we will actually need the result just derived. The reason is that the relative PML incidence is still rising during the third year of treatment (see table 12), ie, the risk for months 37–48 is significantly higher than for months 25–36. However, since the data we will be using is skewed—with the latter period comprising approximately 50% infusions more than the former—to correct this bias, we will assume that the risk during months 37–48 is equal to the (annual) risk for months 49–72. In this way, we will in fact be able to derive a realistic risk estimate for months 25–48 despite the data it is based on being skewed.

We shall proceed in two steps. First we will correct the error in the respective incidence estimate by Biogen Idec as of 5th March 2013, very much like we did above with months 49–72. The resulting estimate of the PML risk is 6.7‰, which is already significantly higher than Biogen Idec's 5.3‰ (ie, is outside the respective 95% confidence interval from [TY-PAN-0597(17) 2014, slide 9]), despite the bias in the exposure data. In the second step we will then rectify the inaccuracy resulting from said bias; our finding is that the actual risk of PML for months 25–48 with JCV-seropositive natalizumab-treated patients who have never used immunosuppressive medication is 7.4‰, ie, about 1 in 135.

For the first step, we basically carry out what we did in §5 for patients with prior immunosuppression. That is, for all infusions administered before April 2011, we shall again assume that across the board, 58.4% of people in question were JCV-positive and that 84% had not had immunosuppression. With all infusions given from April 2011 onwards to patients in the category under consideration (ie, after JCV serology testing became available), we will again use the monthly continuation rates corresponding to the annual dropout rates from table 4 in order to estimate the proportion of patients who were JCV-positive but had not previously used any immunosuppressive agents. All details as well as some more explanations are provided in appendix M; the outcome is that the total exposure was not $24 \times 28\,116 = 674\,784$ patient-months, but only 476 949, which increases the incidence of PML by 41.5%. On the other hand, as with months 49–72, Biogen Idec suppose too low a JCV-prevalence (55% vs 58.4%) and too low a proportion of patients not having had former immunosuppression (79.7% vs 84%). Taking all this into account gives an incidence estimate of

$$5.3‰ \times \frac{674\,784}{476\,949} \times \frac{55\% \times 79.7\%}{58.4\% \times 84\%} = 6.70‰. \qquad (5)$$

However, this estimate is still not entirely realistic, because there is a bias in the exposure data in that 49.3% patient-months more belong to the third year than to the fourth year (285 645 vs 191 304, see table 31 in appendix M), as we remarked earlier. Consequently, the risk of PML seems lower than it actually is, since the relative incidence keeps going up until about the beginning of the fourth treatment year (table 12). We shall now see that it is nevertheless possible to derive a reliable estimate.

Suppose that p_3 is the probability for a JCV-positive individual without prior immunosuppression to develop PML during months 25–36, and let p_4 be the probability for months 37–48. If we set $q_3 = 1 - p_3$ and $q_4 = 1 - p_4$, then the cumulative PML risk for months 25–48 is

$$1 - q_3 q_4$$

as $q_3 q_4$ is the chance of *not* developing PML during this period. However, because of the biased data, what we really obtained in (5) was an estimate of the quantity

$$1 - q_3^{1.2} q_4^{0.8}. \tag{6}$$

As if we solve the system of equations

$$\begin{aligned} e_3 + e_4 &= 2 \\ e_3 &= 1.5 e_4, \end{aligned}$$

then we find that $e_3 = 1.2$ and $e_4 = 0.8$. Here, the first equation is due to the fact that the q's are probabilities concerning a one-year interval but the whole expression actually describes a two-year risk; the second equation ensures that e_3 is 50% greater than e_4, ie, that the exponent (weight) of q_3 is 1.5 times that of q_4.[25] Combining (5) and (6), we get

$$q_3^{1.2} q_4^{0.8} = 1 - 6.7‰ = 0.9933. \tag{7}$$

Now we are almost there—as the relative PML incidence reaches a plateau after three years, we may assume that the risk for the fourth year is the same as with the fifth (or the sixth) year. From the last subsection, we already know that the risk for months 49–72 is 11.0‰, so (recalling that q_4 is an incidence not for 24 months, but for 12 months)

$$q_4 = \sqrt{1 - 11.0‰} = \sqrt{0.989} = 0.994485.$$

Plugging the last expression into (7) we deduce that

$$q_3 = \sqrt[1.2]{\frac{0.9933}{q_4^{0.8}}} = \left(\frac{0.9933}{0.994485^{\frac{4}{5}}} \right)^{\frac{5}{6}} = 0.998087.$$

Thus

$$1 - q_3 q_4 = 1 - 0.994485 \times 0.998087 = 0.007417,$$

ie, the true (unbiased) risk of PML during months 25–48 of natalizumab therapy in JCV-positive patients without past immunosuppression is 7.4‰ or about 1 in 135.

If the reader thought that raising q_3 and q_4 to non-integral powers was strange, here is an alternative way to derive the value of q_3. Suppose that 12 000 JCV-positive patients without prior immunosuppression have received natalizumab for two years already. Assume further that these 12 000 patients take the drug for another year, after which 4 000 individuals decide to stop and the remaining 8 000 continue for one more year. Thus, in this example, the total exposure is equal to $10\,000 \times 24$ patient-months, with the first 12-month period again carrying 50% more weight. If n denotes the number of PML cases in this cohort, then using the (biased) estimate (5), one would expect that

$$n = 10\,000 \times 6.7‰ = 67.$$

On the other hand, in the earlier notation, we also know that

$$n = 12\,000 p_3 + 8\,000 p_4.$$

Since $p_4 = 1 - \sqrt{0.989} = 5.5‰$, we get that

$$p_3 = \frac{67 - 44}{12\,000} = 1.917‰,$$

so that $q_3 = 1 - p_3 = 0.998083$, which is almost exactly the same as the estimate for q_3 of 0.998087 we worked out above using equation (7).

As a final remark, note that the risk really does seem to rise considerably during months 25–48, at least in patients without previous immunosuppression, as the incidence of PML for the third year is just 1.9‰, while for the fourth year it is 5.5‰, ie the risk for months 37–48 is almost three times the risk for months 25–36. Actually, a very similar increase was observed in the STRATA study; there, the PML incidence with months 25–36 was 2.8‰, whereas for months 37–48 it was 7.7‰ (table 15 in appendix E).

§7. Incorporating the JCV antibody index into the PML risk algorithm

This section is concerned with fixing the mistakes in the study [Plavina et al 2014], whose goal was to make the risk estimates for JCV-positive patients with no prior immunosuppression more precise, by also considering titre levels. Unfortunately, for the most part anyway, this goal remains an unaccomplished one, because the only people who indeed do seem to have a lower risk than previously thought are those whose JCV index is at most 0.9—even individuals with an index at or below 1.5 cannot at all be supposed to carry a passable risk, as is falsely suggested ('4-fold lower' [Plavina et al 2014, p 808]). Throughout this section, we shall assume that all patients are JCV-positive with no prior immunosuppressive therapy.

Bounded vs unbounded intervals. The biggest problem with the paper [Plavina et al 2014] was already mentioned in §1, namely, that the authors use intervals that are unbounded below when computing the incidence of PML for patients with a JCV index of up to 1.1 (1.3, 1.5). In this way, Plavina and colleagues totally underestimate the true risks; we will illustrate this through the following example. As stated in [Plavina et al 2014, table 1], about 33.6% of non-PML patients have a JCV index less than or equal to 1.1. Since just three out of 51 (4.4%, according to the authors) patients in the study cohort who developed PML had an index not exceeding 1.1, at first

[25] For the sake of using round numbers we assumed 50% instead of 49.3%, a difference that will be completely immaterial as far as the resulting estimate is concerned.

it appears as if the risk in this group was only about one eighth ($4.4\% \div 33.6\% = 0.13$) of what it would be was there no correlation between JCV titre and risk of PML. However, also from [Plavina et al 2014, table 1], 28.2% of non-PML patients even have a JCV index of at most 0.9, so that only $33.6\% - 28.2\% = 5.4\%$ have an index *between* 0.9 and 1.1. In contrast, two out of 51 PML patients (3.9%) [Plavina et al 2014, figure 4 B] fell into this range, so that the incidence of PML is not reduced by 87%, but merely by 28% ($3.9\% \div 5.4\% = 0.72$).

To put it differently, the real risk of PML for the group of patients with an index between 0.9 and 1.1 is actually 5.5 times as high as Plavina et al claim it is; on further using the realistic PML incidence estimate for months 49–72 of natalizumab treatment in patients without previous exposure to immunosuppression from the last section (11.0‰) as the basis (compared to the 5.4‰ assumed by Plavina et al), the risk is eleven times as high as stated: 7.9‰ (1 in 127) vs 0.7‰ (1 in 1429). Continuing in this fashion, we obtain the following results.

	pts. in this range		
JCV index	non-PML	PML	incidence
≤ 0.9	28.2%	2.0%	0.8‰
> 0.9 and ≤ 1.1	5.4%	3.9%	7.9‰
> 1.1 and ≤ 1.3	4.3%	3.9%	10.0‰
> 1.3 and ≤ 1.5	5.0%	2.0%	4.4‰
> 1.5	57.1%	88.2%	17.0‰
any	100.0%	100.0%	11.0‰

Table 7: Realistic PML incidence estimates for months 49–72 of natalizumab therapy in JCV-positive patients without prior immunosuppression.

The reader may wonder why patients whose JCV index is in the range 1.3–1.5 appear to have a lower risk than those in the range 0.9–1.3. In all likelihood, this just means that there is not yet sufficient data to distinguish between patients in the range 0.9–1.5 as above. So one further learning is that, until more data emerge, all patients with an index between 0.9 and 1.5 should be considered as carrying the same risk. Therefore, all we can say at the moment is the following (the risks for months 25–48 were obtained exactly as above, also using our own incidence estimate from the last section [7.4‰] instead of the 5.2‰ assumed by Plavina et al):

	pts. in this range		risk for months	
JCV index	non-PML	PML	25–48	49–72
≤ 0.9	28.2%	2.0%	0.5‰	0.8‰
0.9–1.5	14.7%	9.8%	4.9‰	7.3‰
> 1.5	57.1%	88.2%	11.4‰	17.0‰
any	100.0%	100.0%	7.4‰	11.0‰

Table 8: Risk estimates by JCV index and natalizumab exposure.

Thus, the risk for people in the range 0.9–1.5 (1 in 204 for months 25–48 and 1 in 137 for months 49–72) is only a third lower compared to the average risk of a JCV-positive patient without prior immunosuppression.

§8. Applications to PML risk-mitigation

Obviously, even better than just having estimates of the PML incidence is to implement policies that may actually prevent the outbreak of PML, without simply taking patients off a highly-effective therapy. And indeed, over the past several years, considerable progress has been made regarding the mitigation of the risks associated with natalizumab administration. Probably the largest such effort is [Ryerson et al 2014], undertaken in America, which as of September 2014 already comprised 861 years of natalizumab exposure among 684 JCV-positive patients who were receiving their infusions not every 28 days, but every 31–61 days, so far without any cases of PML.

Notably, the investigators observed no reduced efficacy at all with these patients: compared to a cohort of 674 individuals on the standard (ie, four-weekly) schedule, all disease-related endpoints—annualised relapse rate, new T2 lesions, new gadolinium-enhancing lesions, proportion of patients without evident disease activity—were essentially identical (marginally more favourable, in fact). In contrast, with the group of people receiving the standard dosage, there were two PML cases. Employing a 'crude but conservative' estimate of the PML incidence (2.4‰ per patient-year), Ryerson and colleagues calculated that 1248 years of exposure free from PML are necessary to accomplish statistical significance at the 5% confidence level. However, using the actuarial risks from table 11 in appendix B, we will now see that no further data are actually needed, ie, the p-value for these findings already is below 0.05.

As just outlined, in the study being considered (which is still ongoing), there are two arms: one for patients on the standard dose (SD, $n = 674$), and the other for those deploying extended dosing (ED, $n = 684$). Importantly, as also mentioned already, so far no-one in the ED group developed PML, while two patients in the SD group did, in spite of the fact that as of September 2014, the JCV-seroprevalence in the latter group was significantly lower (46% vs 66%), as was the mean number of natalizumab infusions administered (24.4 vs 39.6). What is more, the ED arm itself consists of three subgroups:

- EED ('early extended dose only'), one infusion every 31–48 days;
- LED ('late extended dose only'), one infusion every 49–61 days;
- VED ('variable extended dose'), where people utilised both the EED and the LED schedules, for varying durations.

We will proceed by separately computing the p-value for each of these three subgroups; their product is then below 0.05. The steps involved are always the same—we shall first 'convert' the number of patients to the equivalent number in the US post-marketing setting (ie, by taking account of the differences in JCV-positivity and prior use of immunosuppressants); using the actuarial incidences for the US in appendix B, we will hence estimate the odds of not developing PML (which are different for each subgroup of the ED cohort as the mean total number of natalizumab infusions as well as the average duration of the ED schedule varied between groups). All data we will be using is as of September 2014, and may be found in [Ryerson et al 2014, table 1].

With the EED group ($n = 231$), 62% of patients were JCV-positive with 10% having had prior immunosuppression (vs 55% respectively 14% of US post-marketing patients). Conservatively supposing that individuals with previous immunosuppression have three times the risk, these 231 study patients bear the joint burden of about

$$231 \times \frac{62\%}{55\%} \times \frac{90\% \times p + 10\% \times 3p}{86\% \times p + 14\% \times 3p} = 244$$

US post-marketing patients, where p is the average risk of a JCV-positive US post-marketing patient. (The numerical value of p is unimportant as all p's cancel out.)

As mentioned already, as per the study protocol, being a member of the EED group meant receiving one infusion every 31–48 days, so that the average cycle length was approximately 39 days. Moreover, the average number of infusions in this group was 49, while the average duration of the ED schedule was 17 months. Assuming the above cycle length of 39 days, 17 months of extended dosing correspond to about $(17 \times 30) \div 39 = 13$ infusions on ED. Thus, on average, patients in the EED group were given $49 - 13 = 36$ four-weekly doses before switching to longer intervals. Therefore we will now calculate the likelihood of not developing PML in America between the 37th and the 54th infusion, inclusive. (Because 17 months of natalizumab treatment beyond the 36th infusion would usually entail receiving 18 infusions more.) That is, in the notation of table 11 in appendix B we need to compute

$$q_{EED} := q_{37} q_{38} \cdots q_{54}.$$

However, since the probabilities in this table only go out to infusion no. 48, we shall assume that the risk for infusions 49–54 is the same as with infusions 43–48 (as we do in appendix C), which is justified because in the US, the relative PML risk reaches its plateau by the middle of the fourth year of natalizumab treatment (table 12). Thus, using the data for America from table 11,

$$\begin{aligned} q_{EED} &= q_{37} q_{38} \cdots q_{48} \times q_{43} q_{44} \cdots q_{48} \\ &= \frac{q_1 q_2 \cdots q_{48}}{q_1 q_2 \cdots q_{36}} \times \frac{q_1 q_2 \cdots q_{48}}{q_1 q_2 \cdots q_{42}} \\ &= \frac{0.995541^2}{0.997806 \times 0.996926} \\ &= 0.9963. \end{aligned}$$

Therefore the chance of no PML in the EED group is

$$q_{EED}^{244} = 0.9963^{244} = 0.4048. \tag{8}$$

Next we will calculate the p-value for the LED group ($n = 245$). In this group, 67% of all patients were JCV-positive, with 31% having had prior immunosuppressive therapy. Because the past use of immunosuppressants in the LED cohort is actually higher than in post-marketing, again so as to be conservative, this time we will assume that individuals with former immunosuppression have just double the risk of PML, so that these 245 study subjects correspond to

$$245 \times \frac{67\%}{55\%} \times \frac{69\% \times p + 31\% \times 2p}{86\% \times p + 14\% \times 2p} = 343$$

US post-marketing patients. For with the LED group, individuals were administered one natalizumab dose every 49–61 days, the average cycle length was about 55 days. Furthermore, the average number of doses was 32, and the average duration of the ED schedule was 24 months (again corresponding to $(24 \times 30) \div 55 = 13$ infusions on ED, coincidentally), so that these patients were given an average of 19 four-weekly natalizumab cycles before starting ED. Hence we will now work out the odds of a US post-marketing patient to develop PML during natalizumab infusions 20–45, as 24 months of therapy beyond the 19th infusion would normally have meant another 26 infusions. Therefore we need to compute

$$q_{LED} := q_{20} q_{21} \cdots q_{45}.$$

Again using the US data from table 11 we have

$$\begin{aligned} q_{LED} &= \frac{q_1 q_2 \cdots q_{45}}{q_1 q_2 \cdots q_{19}} \\ &= \frac{0.995938}{0.999934} \\ &= 0.9960. \end{aligned}$$

So the probability of not a single case of PML in the LED group is

$$q_{LED}^{343} = 0.9960^{343} = 0.2529. \tag{9}$$

We carry out the same procedure once more, for the VED subgroup ($n = 208$), 69% of whose members were

JCV-positive with 17% having formerly received immunosuppressive drugs. Thus, these 208 individuals carry the risk of about

$$208 \times \frac{69\%}{55\%} \times \frac{83\% \times p + 17\% \times 2p}{86\% \times p + 14\% \times 2p} = 268$$

US post-marketing patients. Because those in the VED group used, at various times, both EED and LED dosing, we will assume that the average VED natalizumab cycle length is the average of that for the other two groups, ie, $(39 + 55) \div 2 = 47$ days. As the average number of infusions in VED subjects was 37 and the average duration of the ED schedule was 26 months (which corresponds to $(26 \times 30) \div 47 = 17$ infusions while on ED), these people had received an average of $37 - 17 = 20$ four-weekly infusions before choosing to use longer dosing intervals. In the by now familiar notation we therefore have

$$q_{VED} = q_{21} q_{22} \cdots q_{48},$$

because 26 additional months of natalizumab therapy beyond the 20th infusion would normally involve another 28 infusions. Using the probabilities in table 11 we get

$$\begin{aligned} q_{VED} &= \frac{q_1 q_2 \cdots q_{48}}{q_1 q_2 \cdots q_{20}} \\ &= \frac{0.995541}{0.999934} \\ &= 0.9956. \end{aligned}$$

Hence the p-value of no PML cases with VED is

$$q_{VED}^{268} = 0.9956^{268} = 0.3067. \qquad (10)$$

Finally, using (8) and (9), the overall p-value of no PML cases with all three ED subgroups is therefore

$$0.4048 \times 0.2529 \times 0.3067 = 0.0314,$$

ie, the fact that there were no PML cases whatsoever is indeed statistically significant. Note also that the number of PML cases one would have expected for the three ED subgroups combined is

$$\begin{aligned} (1 - q_{EED}) \times 244 + (1 - q_{LED}) \times 343 & \\ +(1 - q_{VED}) \times 268 &= \\ 3.7‰ \times 244 + 4‰ \times 343 + 4.4‰ \times 268 &= 3.5. \end{aligned}$$

So the conclusion is that, even on assuming one hypothetical PML case in an ED patient, it appears as if it is possible to cut the risk of PML by at least two thirds, simply by extending the dosing intervals. In fact, the expected number of PML cases is in reality probably greater than 3.5, because the above does not take into account that the average JCV index in the ED group was also significantly higher than in the SD group (1.6 vs 0.83, $p < 0.01$). Thus it is quite likely that just within the scope of the trial [Ryerson et al 2014], the strategy of using extended dosing already prevented four PML cases in fewer than 500 JCV-positive natalizumab-treated patients—without compromising treatment efficacy. Actually, from a conceptual standpoint, too, it is perfectly plausible that this measure might work; in discussing the paper [Harrer et al 2015], Gavin Giovannoni commented that if 'the MSers with wearing-off have a lower, or no, risk of PML, but still have a excellent, or good, therapeutic response to natalizumab may be we could optimise the dose of natalizumab to de-risk natalizumab and prevent PML' [Giovannoni 2015c].

§9. How the PML incidences should be calculated: the actuarial method

As explained already, the flaw in Biogen Idec's approach to assessing PML incidences is that it fails to consider that natalizumab exposure varies substantially even in patients who belong to the same category (same combination of risk factors and same interval of treatment). Technically, Biogen Idec are computing what is called an *absolute risk*—they simply divide the number of PML cases that have occurred in a given category by the number of people ever having belonged to that category. So far, so good. The problem is, the resulting incidences are then communicated as if they had been obtained using the actuarial method. The latter, however, in fact takes into account the duration of therapy for each individual, as well as that risks may change over time, and thereby yields much more precise, and in the case of natalizumab, much higher, estimates than the 'absolute' formula.

As we will see very shortly, the actuarial method is still quite easy to apply. Its main drawback is that it requires the raw PML data, ie, in order to properly estimate the incidence of PML for a given treatment interval, it is not sufficient to just know the total number of PML cases that have occurred in the period in question; rather, for each case, one needs to know exactly how long the respective individual had been on natalizumab at the time PML developed. Since the raw PML data are no longer disclosed by Biogen Idec, only their own statisticians are able to compute the PML incidences correctly (using recent data, see appendix B).

We shall now describe in detail the actuarial method for calculating risks. We will once more proceed by example. Suppose that we wish to estimate the PML incidence during the third year of natalizumab therapy.[26] Assume that for each $i = 25, 26, \ldots, 36$, we are given that n_i patients have received natalizumab for at least i months, and among those, m_i individuals developed PML during month i of therapy. So the risk for month i is $m_i : n_i$;

[26] Of course, one may also restrict oneself to a subgroup of patients, eg, to all patients who are further positive for JCV antibodies but have not had prior immunosuppression.

we denote this quantity by p_i, ie, we set $p_i = \frac{m_i}{n_i}$. Accordingly, the probability of not developing PML during month i is $q_i := 1 - p_i$. Hence the chance of making it through the whole of the third year without getting PML is $q_{25}q_{26}\cdots q_{36}$, and so the odds of developing PML at some point during the third year of natalizumab treatment are finally

$$1 - q_{25}q_{26}\cdots q_{36}. \tag{11}$$

Actually, when the p's involved are small (as is the case with natalizumab, since the chance of developing PML during a particular month of therapy is usually less than 1 : 1000), the last expression is roughly equal to

$$p_{25} + p_{26} + \cdots + p_{36}.^{27} \tag{12}$$

For instance, if $p_{25} = p_{26} = \cdots = p_{36} = 1‰$, then their sum equals 12‰ while the exact estimate, obtained from (11), is $1 - 0.999^{12} = 11.93‰$. Going back to the real-life example from §3 (p 7), what Bloomgren et al should have calculated to realistically estimate the risk of PML for months 25–48 in JCV-positive natalizumab-treated patients with prior immunosuppression is therefore

$$\frac{m_{25}}{4681} + \frac{m_{26}}{4534} + \cdots + \frac{m_{47}}{1707} + \frac{m_{48}}{1585},$$

but what they actually computed was

$$\frac{m_{25}}{4681} + \frac{m_{26}}{4681} + \cdots + \frac{m_{47}}{4681} + \frac{m_{48}}{4681}.$$

Comparing these two expressions, we see that, especially with the last few terms, the denominators used by Bloomgren and coauthors are too big, hence the respective fractions are too small, and consequently the sum of all these quantities underestimates the true risk.

Using data of July, 2011, in appendix B, we once more explicitly show the differences between the incidences calculated the actuarial way and the absolute way (see also table 2).

§10. L-selectin and lipid-specific IgM bands

In this section, we will discuss two breakthrough advances in the stratification of natalizumab-treated MS patients, L-selectin (CD62L) [Schwab et al 2013] and lipid-specific IgM bands in cerebrospinal fluid [Villar et al 2015]. The latter is a static parameter and therefore has to be evaluated once only. In contrast, the former monitors the actual pharmacodynamics of natalizumab and so needs to be assessed regularly during long-term application. And a dynamic marker is what was urgently required—in reviewing [Harrer et al 2015], Gavin Giovannoni remarked that this study demonstrates that natalizumab 'does not have the same effect on the immune system in everyone' and 'shows that levels of the drug vary between individuals and the effect on immune surveillance of the central nervous system differs depending on levels of natalizumab on the surface of cells ... There is only one certainty about nature and it is that it is highly variable; so I am not surprised that a fixed dose of a drug ... will have different effects on different people' [Giovannoni 2015c].

Given that L-selectin and lipid-specific IgM bands seem to be well-known and their usefulness accepted within the community already, it is quite astonishing that these two tests still have not entered routine clinical practice. One might point out that neither biomarker has been verified prospectively so far, ie, that more data are needed until their use may be recommended. But is that really true? Let us take a look at the numbers. However, before we begin, recall that around the time JCV serology testing was made generally available, the respective ELISA had also not been verified prospectively yet. Rather, the decision to officially inaugurate JCV testing was based on the retrospective examination of 31 natalizumab-treated patients with PML for whom blood samples obtained at least six months ahead of PML development were available; all samples tested positive with regard to JCV antibodies [Kappos et al 2011a, p 749]. Given the 55% JCV-seroprevalence of the original two-step assay, the p-value of this finding is

$$0.55^{31} \approx 9 \times 10^{-9}. \tag{13}$$

In comparison, the eight pre-PML serum samples from [Schwab et al 2013], all of which exhibited an exceptionally low percentage of CD62L-expressing $CD4^+$ T-cells, correspond to a p-value of

$$0.066^8 \approx 4 \times 10^{-10}, \tag{14}$$

since only approximately 6.6% of long-term natalizumab-treated patients are considered to have critically low L-selectin levels [Schwab et al 2014]. Observe that (14) is even smaller than (13); in fact,

$$0.066^8 < 0.55^{36},$$

ie, eight CD62L-low PML patients are statistically as significant as 36 JCV-positive PML patients. Another way to calculate a perhaps slightly more precise p-value for the association of L-selectin with a patient's future risk of getting PML is to use Fisher's exact test. In the cohort from [Schwab et al 2014], 182 of 273 subjects (66.5%) either had a JCV index exceeding 0.9 or were JCV-seropositive and had previously taken immunosuppressants. Among these 182, merely 12 (6.6%) had CD62L values below the assay threshold vs all eight PML patients from the original trial [Schwab et al 2013] for whom pre-PML blood samples were available. Alas, comparing these two populations using standard statistical software packages

[27] If a 12-month period is considered and all p's are less than or equal to 1/1000, then the difference between the estimates from the earlier formula (11) and that of the simpler expression (12) is below 0.1‰. Even if the period in question is 24 months, the error is still less than 0.3‰.

(usually) only gives that $p < 0.0001$. Fortunately, in this particular case—as there were no false-negatives and the numbers are relatively small—it is easy to carry out the computations involved by hand; after all, Fisher's exact test really just answers the question: what is the chance that in a random sample of size eight drawn from a set of 190 people of whom 20 are positive for a certain characteristic, all eight in the sample are positive? This can be worked out using a very elementary probabilistic calculation using binomial coefficients only; we have

$$\begin{aligned} p &= \frac{\binom{20}{8}\binom{170}{0}}{\binom{190}{8}} \\ &= \frac{\frac{20!}{8!\times 12!}}{\frac{190!}{8!\times 182!}} \\ &= \frac{20!\times 182!}{12!\times 190!} \\ &= \frac{13\times 14\times\cdots\times 20}{183\times 184\times\cdots\times 190} \\ &\approx 3.5\times 10^{-9}, \end{aligned}$$

which is also less than (13). So already a year ago, there was more than enough evidence to justify the introduction of the CD62L marker into medical practice. In fact, from *Medscape*, by September 2014, there were 15 natalizumab-treated PML patients whose L-selectin levels had been screened; 14 were remarkable prior to the diagnosis of PML [Hughes 2014]. Because only about 10% of the more than 1000 subjects in the cohort in question were CD62L-low, the resulting p-value is hence

$$0.1^{14}\times\binom{15}{1}\times 0.9 < 0.55^{49}.$$

Thus, despite one false-negative case, these findings are as relevant as 49 natalizumab-treated PML patients who were all JCV-seropositive. Lastly, again from *Medscape*, one distinctive feature of L-selectin expression—which is not captured in the above inequality—is that it also decreases with the duration of natalizumab treatment and that the decrease is twice as high in patients with prior immunosuppression, in line with the fact that the PML risk increases with time on therapy and that individuals who have formerly been exposed to immunosuppressants have about twice the risk as those not having used such drugs [Keller 2014].

We will now compute the p-value for the cohort tested for lipid-specific IgM bands [Villar et al 2015], also using a Fisher's exact test. Funnily, the result is exactly what we got when we applied this test to the data about the L-selectin marker. By [Villar et al 2015, table 4], in total there were 176 JCV-seropositive natalizumab-treated patients with MS, including 23 individuals who developed PML. Of note, though 105 subjects (60%) were positive for lipid-specific IgM bands, just one PML patient was; the remaining 22 were among the 71 lacking said bands. So in the abstract, we wish to compare two populations consisting of 23 respectively 153 members such that for the first group, all but one individual (96%) possess the quality that we are interested in, whereas in the second, only 49 (32%) do. Hence the p-value for Fisher's exact test in this situation is

$$\begin{aligned} p &= \frac{\binom{71}{23}\binom{105}{0}+\binom{71}{22}\binom{105}{1}}{\binom{176}{23}} \\ &= \frac{\frac{71!}{23!\times 48!}+\frac{71!}{22!\times 49!}\times 105}{\frac{176!}{23!\times 153!}} \\ &= \frac{\frac{49\times 50\times\cdots\times 71}{1\times 2\times\cdots\times 23}+\frac{50\times 51\times\cdots\times 71}{1\times 2\times\cdots\times 22}\times 105}{\frac{154\times 155\times\cdots\times 176}{1\times 2\times\cdots\times 23}} \\ &= \frac{49\times\cdots\times 71+50\times\cdots\times 71\times 23\times 105}{154\times 155\times\cdots\times 176} \\ &\approx \frac{7\times 10^{40}+3.4\times 10^{42}}{9.9\times 10^{50}} \\ &= \frac{3.47}{9.9\times 10^{8}} \\ &\approx 3.5\times 10^{-9}. \end{aligned}$$

Let us break down the first equality, namely

$$p = \frac{\binom{71}{23}\binom{105}{0}+\binom{71}{22}\binom{105}{1}}{\binom{176}{23}}.$$

Here, the denominator equals the number of ways to select 23 individuals from a universe of 176 candidates; in the numerator, we are computing the number of possibilities such that the 23 chosen contain at most one false-negative, ie, either no false-negatives (the first term), or exactly one false-negative (the second term).

In conclusion, both CD62L and the lipid-specific IgM bands of course do need to be verified prospectively, no doubt about that. All the same, the p-values established in this section speak for themselves, so it will be hard to argue why these two tests should be withheld for much longer. In the author's opinion anyway, as of today, any JCV-seropositive natalizumab-treated patient who wants their values to be checked out ought to be able to do so, free of charge.

A The evolution of the estimates of the incidences of natalizumab-associated PML in JCV-positive patients with multiple sclerosis

	no prior IS treatment		prior IS treatment		
estimates as of	mo. 1–24	mo. 25–48	mo. 1–24	mo. 25–48	reference
March 2011	0.35‰	2.5‰	1.2‰	7.8‰	[Kappos et al 2011a, figure 3]
February 2012	0.56‰	4.6‰	1.6‰	11.1‰	[Bloomgren et al 2012, figure 2 B]
September 2012	0.6‰	5.2‰	1.8‰	10.6‰	[TY-PAN-0587(1) 2013, slide 17]
March 2013	0.7‰	5.3‰	1.8‰	11.2‰	[TY-PAN-0597(16) 2014, slide 10]

Table 9: How Biogen Idec's PML risk estimates in JCV-positive natalizumab users have evolved over time.

B An example showing the difference between the Biogen Idec and the actuarial methods, using the first 143 cases of PML on natalizumab

As mentioned already, Biogen Idec do not publish the raw PML data anymore. Fortunately, the slides of a talk by Brigitte Keller-Stanislawski [Keller-Stanislawski 2011] contain all the information necessary to estimate the risk of PML the actuarial way: slide 6 of this talk has a bar chart that shows exactly how many PML cases there were in patients with MS after $1, 2, \ldots, 54$ months of natalizumab therapy, as well as the countries where each case occurred; slide 7 further provides incidence estimates from which one may recover the actual number of patients by duration of treatment. (All data are as of early July 2011.) We thus obtain the following table:

	US			EEA/ROW		
exposure	PML cases	PML incidence	patients	PML cases	PML incidence	patients
overall	57	1.045‰	54 545	86	2.073‰	41 486
6+ months	57	1.372‰	41 545	86	2.407‰	35 729
12+ months	57	1.734‰	32 872	84	2.798‰	30 021
18+ months	55	2.064‰	26 647	75	3.029‰	24 761
24+ months	47	2.326‰	20 206	67	3.343‰	20 042
30+ months	32	2.157‰	14 835	41	2.620‰	15 649
36+ months	20	1.519‰	$*$	20	1.931‰	10 357
42+ months	14	2.038‰	6869	12	1.710‰	7018
48+ months	6	1.261‰	4758	3	0.939‰	3195

Table 10: Natalizumab exposure data as of July 2011.

(The '$*$' indicates that the respective incidence appears implausible, and was therefore omitted when interpolating patient numbers.) For both the US and the EEA/ROW portions of the table, the first ('PML cases') and second columns ('PML incidence') are really just slide 6 respectively slide 7 from [Keller-Stanislawski 2011]. The values in the second column were originally calculated using the formula

$$p = \frac{m}{n},$$

where m is the number of PML cases, n is the number of patients, and p is the resulting incidence of PML. Solving for n yields

$$n = \frac{m}{p},$$

ie, given p (the PML incidence) and m (the number of PML cases), one can compute n (the number of patients). So, for example, the number of US-patients with at least 24 months of natalizumab therapy as of early July 2011 was $47 \div 0.002326 = 20\,206$. The next table finally gives the actuarial risk (see §7) of developing PML during therapy with natalizumab, where the missing patient numbers were obtained using cubic spline interpolation:

	US					EEA/ROW				
month	cases	patients	q_i	$q_1 q_2 \cdots q_i$	cum. in.	cases	patients	q_i	$q_1 q_2 \cdots q_i$	cum. in.
1	0	54 545	1.000000	1.000000	0.00‰	0	41 486	1.000000	1.000000	0.00‰
2	0	51 344	1.000000	1.000000	0.00‰	0	40 226	1.000000	1.000000	0.00‰
3	0	48 471	1.000000	1.000000	0.00‰	0	39 025	1.000000	1.000000	0.00‰
4	0	45 898	1.000000	1.000000	0.00‰	0	37 879	1.000000	1.000000	0.00‰
5	0	43 598	1.000000	1.000000	0.00‰	0	36 782	1.000000	1.000000	0.00‰
6	0	41 545	1.000000	1.000000	0.00‰	0	35 729	1.000000	1.000000	0.00‰
7	0	39 710	1.000000	1.000000	0.00‰	0	34 714	1.000000	1.000000	0.00‰
8	0	38 068	1.000000	1.000000	0.00‰	1	33 732	0.999970	0.999970	0.03‰
9	0	36 590	1.000000	1.000000	0.00‰	1	32 777	0.999969	0.999940	0.06‰
10	0	35 249	1.000000	1.000000	0.00‰	0	31 843	1.000000	0.999940	0.06‰
11	0	34 019	1.000000	1.000000	0.00‰	0	30 927	1.000000	0.999940	0.06‰
12	0	32 872	1.000000	1.000000	0.00‰	1	30 021	0.999967	0.999907	0.09‰
13	1	31 783	0.999969	0.999969	0.03‰	1	29 122	0.999966	0.999872	0.13‰
14	0	30 735	1.000000	0.999969	0.03‰	2	28 230	0.999929	0.999801	0.20‰
15	0	29 713	1.000000	0.999969	0.03‰	1	27 346	0.999963	0.999765	0.24‰
16	1	28 701	0.999965	0.999934	0.07‰	1	26 473	0.999962	0.999727	0.27‰
17	0	27 684	1.000000	0.999934	0.07‰	3	25 610	0.999883	0.999610	0.39‰
18	0	26 647	1.000000	0.999934	0.07‰	1	24 761	0.999960	0.999570	0.43‰
19	0	25 580	1.000000	0.999934	0.07‰	2	23 926	0.999916	0.999486	0.51‰
20	0	24 491	1.000000	0.999934	0.07‰	0	23 107	1.000000	0.999486	0.51‰
21	5	23 396	0.999786	0.999720	0.28‰	3	22 308	0.999866	0.999352	0.65‰
22	1	22 307	0.999955	0.999675	0.32‰	1	21 529	0.999954	0.999305	0.69‰
23	2	21 239	0.999906	0.999581	0.42‰	1	20 773	0.999952	0.999257	0.74‰
24	3	20 206	0.999852	0.999433	0.57‰	8	20 042	0.999601	0.998858	1.14‰
25	4	19 219	0.999792	0.999225	0.78‰	4	19 336	0.999793	0.998652	1.35‰
26	2	18 275	0.999891	0.999115	0.88‰	2	18 641	0.999893	0.998544	1.46‰
27	1	17 370	0.999942	0.999058	0.94‰	5	17 941	0.999721	0.998266	1.73‰
28	2	16 499	0.999879	0.998937	1.06‰	2	17 220	0.999884	0.998150	1.85‰
29	3	15 656	0.999808	0.998745	1.25‰	5	16 461	0.999696	0.997847	2.15‰
30	1	14 835	0.999933	0.998678	1.32‰	1	15 649	0.999936	0.997783	2.22‰
31	1	14 033	0.999929	0.998607	1.39‰	3	14 774	0.999797	0.997581	2.42‰
32	3	13 251	0.999774	0.998381	1.62‰	5	13 859	0.999639	0.997221	2.78‰
33	4	12 490	0.999680	0.998061	1.94‰	7	12 930	0.999459	0.996681	3.32‰
34	3	11 752	0.999745	0.997806	2.19‰	1	12 018	0.999917	0.996598	3.40‰
35	0	11 038	1.000000	0.997806	2.19‰	4	11 151	0.999641	0.996240	3.76‰
36	0	10 350	1.000000	0.997806	2.19‰	4	10 357	0.999614	0.995856	4.14‰
37	0	9690	1.000000	0.997806	2.19‰	0	9657	1.000000	0.995856	4.14‰
38	2	9060	0.999779	0.997586	2.41‰	1	9041	0.999889	0.995746	4.25‰
39	1	8461	0.999882	0.997468	2.53‰	0	8488	1.000000	0.995746	4.25‰
40	1	7895	0.999873	0.997342	2.66‰	1	7979	0.999875	0.995621	4.38‰
41	2	7364	0.999728	0.997071	2.93‰	2	7496	0.999733	0.995355	4.64‰
42	1	6869	0.999854	0.996926	3.07‰	3	7018	0.999573	0.994930	5.07‰
43	3	6412	0.999532	0.996459	3.54‰	2	6527	0.999694	0.994625	5.38‰
44	1	5995	0.999833	0.996293	3.71‰	1	6003	0.999833	0.994459	5.54‰
45	2	5619	0.999644	0.995938	4.06‰	1	5427	0.999816	0.994276	5.72‰
46	1	5287	0.999811	0.995750	4.25‰	1	4780	0.999791	0.994068	5.93‰
47	0	4999	1.000000	0.995750	4.25‰	1	4042	0.999753	0.993822	6.18‰
48	1	4758	0.999790	0.995541	4.46‰	1	3195	0.999687	0.993511	6.49‰

Table 11: Actuarial risk of natalizumab-associated PML in patients with MS, estimated using post-marketing data of July 2011.

For both the US and the EEA/ROW portions, the first column has the number of PML cases that, as of July 2011, had occurred during month i, $1 \leq i \leq 48$, the second column the number of patients that had reached that month of natalizumab therapy, the third column the chance of not developing PML during that month only, the fourth column the chance of not developing PML during months $1, \ldots, i$, inclusive, and the fifth column the probability of the complement event, ie, the risk for months $1, \ldots, i$ (the cumulative PML incidence for the first i months of natalizumab treatment).

As an example, in the US, the actuarial risk during the first 24 months is 0.57‰ (1 : 1754); with the same

constellation, the Biogen Idec risk is 13 : 54545, ie, 0.24‰ (1 : 4196). Even for the next 24 months—in which the relative risk does not rise nearly as much as during the first 24 months (table 12 below)—the discrepancy remains considerable: 3.89‰ (1 : 257) vs 2.03‰ (1 : 493), ie, the true incidence of PML is still almost double the Biogen Idec incidence.

The following shows the PML incidences for each trimester of the first four years of natalizumab therapy. Note that, in the EEA/ROW, the incidence peaks during the last trimester of the third year of treatment, while in the US, the incidence peaks only in the second trimester of the fourth year:

	US		EEA/ROW		worldwide	
trimester	$q_i q_{i+1} q_{i+2} q_{i+3}$	incidence	$q_i q_{i+1} q_{i+2} q_{i+3}$	incidence	$q_i q_{i+1} q_{i+2} q_{i+3}$	incidence
1/year 1	1.000000	0.00‰	1.000000	0.00‰	1.000000	0.00‰
2/year 1	1.000000	0.00‰	0.999970	0.03‰	0.999986	0.01‰
3/year 1	1.000000	0.00‰	0.999936	0.06‰	0.999970	0.03‰
1/year 2	0.999934	0.07‰	0.999820	0.18‰	0.999879	0.12‰
2/year 2	1.000000	0.00‰	0.999759	0.24‰	0.999884	0.12‰
3/year 2	0.999499	0.50‰	0.999373	0.63‰	0.999435	0.57‰
1/year 3	0.999504	0.50‰	0.999291	0.71‰	0.999396	0.60‰
2/year 3	0.999444	0.56‰	0.999068	0.93‰	0.999252	0.75‰
3/year 3	0.999425	0.57‰	0.998632	1.37‰	0.999026	0.97‰
1/year 4	0.999534	0.47‰	0.999764	0.24‰	0.999649	0.35‰
2/year 4	0.998947	1.05‰	0.998833	1.17‰	0.998890	1.11‰
3/year 4	0.999245	0.75‰	0.999047	0.95‰	0.999168	0.83‰

Table 12: How the relative PML incidence changes over time in the US vs the EEA/ROW.

C Computing the p-values that establish that a drug holiday reduces the risk of PML

We here supply the details of the computation that demonstrates that the data from the STRATA trial available in [O'Connor et al 2014] are sufficient evidence that a drug holiday does reduce the PML incidence. We will proceed by first estimating the odds of not developing PML during months 25–48 of natalizumab therapy with STRATA participants (but using the post-marketing PML incidences as a starting point). We will hence derive the p-value of nobody developing PML in the first two years of STRATA—as was in fact the case—recalling that all participants of this study had already been exposed to natalizumab prior to enrolment, receiving 32 infusions on average, but assuming that the 57+ week drug holiday everybody had to take before STRATA did not actually change the infusion counter. We shall then repeat these steps for months 25–54 of natalizumab treatment, since there were in fact no cases of PML during the first 30 months in STRATA.

From appendix B, the chance of not developing PML in the US during months 25–48 of natalizumab therapy is (in the notation of the table 11)

$$q_{25} q_{26} \cdots q_{48} = \frac{q_1 q_2 \cdots q_{48}}{q_1 q_2 \cdots q_{24}} = \frac{0.995541}{0.999433} = 0.99611. \tag{15}$$

(So the PML incidence in this constellation is $1 - 0.99611 = 3.89‰$.) Similarly the probability of no PML in a natalizumab-treated patient in the EEA/ROW for the same period is

$$\frac{0.993511}{0.998858} = 0.99465. \tag{16}$$

(Corresponding to an incidence of 5.35‰.)

As is shown in figure e-3 in [O'Connor et al 2014], as of August 2013, there were 752 individuals in STRATA with at least 24 natalizumab infusions. By figure 1 in the same reference, 356 out of 1094 STRATA patients were based in the US. Assuming this proportion, of the 752 subjects with 24+ infusions, 245 were in the US and 507 were in the EEA/ROW. However, because JCV-seroprevalence (67%) is higher in STRATA than in post-marketing but the prior use of immunosuppression (7%) is lower, we once more need to apply a correction factor, very much like (1) in §2, except that we will use separate factors for the US and the EEA/ROW.

From [Bloomgren et al 2012], in the US, 51% of patients are supposed to be JCV-positive while the prior IS use is estimated at 14%. Hence the US correction factor is (see p 4)

$$\frac{67\% \times (7\% \times 3 + 93\%)}{51\% \times (14\% \times 3 + 86\%)} = 1.170,$$

where we conservatively assumed that someone with prior IS therapy has three times the risk of a comparable non-IS individual. Therefore, 245 US-subjects from STRATA carry the PML risk of about 287 post-marketing US-patients, and hence, using (15), the chance of no PML in this group is

$$0.99611^{287} = 0.32673. \tag{17}$$

Similarly, since as per [Bloomgren et al 2012], for the EEA/ROW, the proportion of JCV-positive patients is estimated to be 59% while 23.5% prior IS use is assumed, the resulting correction factor is

$$\frac{67\% \times (7\% \times 3 + 93\%)}{59\% \times (23.5\% \times 3 + 76.5\%)} = 0.881,$$

so that in the EEA/ROW, 507 STRATA patients carry about the same risk as 447 post-marketing patients. Using (16), the probability of no PML cases in these 507 patients (with 24+ infusions in STRATA) is

$$0.99465^{447} = 0.09091.$$

Multiplying this p-value and the one calculated for America (17) together yields an overall p-value for the event of no PML cases during the first 24 months in the above 752 STRATA patients of

$$0.32673 \times 0.09091 = 0.02970.$$

So this result is statistically significant, but of course it was obtained under the hypothesis that the drug holiday the STRATA subjects had taken did not lower their PML risk when natalizumab was later restarted. Thus we may reject this hypothesis. In fact, as mentioned already, even during the first 30 months (not just the first 24 months) of natalizumab in STRATA, there were no cases of PML. Therefore we shall now also calculate the p-value of this event. We will do this in a very similar manner, ie, we will first estimate the chance of not developing PML during months 25–54 in post-marketing. Since the cumulative probabilities of no PML in table 11 go out to month 48 of therapy only, we will assume that the risk for months 49–54 is the same as for months 43–48. Hence our estimate of the chance of no PML in the US with months 25–54 is

$$(q_{25}q_{26}\cdots q_{48})(q_{43}q_{44}\cdots q_{48}) = \frac{q_1q_2\cdots q_{48}}{q_1q_2\cdots q_{24}} \times \frac{q_1q_2\cdots q_{48}}{q_1q_2\cdots q_{42}} = \frac{0.995541}{0.999433} \times \frac{0.995541}{0.996926} = 0.99472. \tag{18}$$

Likewise, for the EEA/ROW, the respective probability is

$$\frac{0.993511}{0.998858} \times \frac{0.993511}{0.994930} = 0.99323. \tag{19}$$

From [O'Connor et al 2014, figure e-3], as of August 2013, 724 patients had received 30+ infusions in STRATA. If we again assume that 32.54% of these are based in America, then this works out to 236 US-subjects vs 488 EEA/ROW-subjects. Applying the respective correction factors from above, we see that in the US, 236 STRATA participants have the risk of about 276 post-marketing patients while in the EEA/ROW, 488 STRATA participants correspond, risk-wise, to 430 post-marketing patients. Putting it all together, ie, using the probabilities (18) and (19), the event of no PML in the 724 STRATA patients with at least 30 infusions has a chance of

$$0.99472^{276} \times 0.99323^{430} = 0.23197 \times 0.05388 = 0.01250.$$

So this is an even more significant finding, which makes it even less probable that a drug holiday is not helpful.

Lastly, another circumstance that makes these results yet more relevant—but is somewhat hard to quantify—is the fact that the PML incidence in JCV-positive patients after 32 months of therapy, estimated the actuarial way, is higher in STRATA (see table 11 and table 15), even when compared only to that in EEA/ROW-patients. Therefore, no PML cases in STRATA is a stronger finding than no PML cases in post-marketing during the same treatment interval. (Recall that the calculations in this section are based on post-marketing PML incidences.)

D Correctly estimating the number of patient-months

The table below demonstrates how to correctly estimate the total experience (number of patient-months) in the cohort analysed in [Bloomgren et al 2012] for the third and fourth years of natalizumab treatment in individuals who are JCV-positive and have had prior IS treatment.

The second column shows the (unrealistic) patient numbers assumed by Bloomgren and coauthors. For the other three columns (the 'correct' portion of the table), we used that, at the time of the analysis by Bloomgren et al, there were 45 533 patients with at least 25 months of natalizumab therapy, but only 29 085 and 14 239 patients with at least 37 months respectively 49 months [Bloomgren et al 2012, figure 1]; these numbers then further need to be multiplied by the appropriate factor. So, for instance, for the second column, 18.69% (14% in the US, 23.5% in the EEA/ROW) of all JCV+ patients were assumed to have had prior immunosuppression. Therefore, in all, there were

$$29\,085 \times 55\% \times 18.69\% = 2990$$

JCV+ patients with prior IS treatment and at least 37 months of natalizumab exposure. Similarly, there were

$$14\,239 \times 55\% \times 18.69\% = 1464$$

JCV+, IS+ patients with at least 49 months; the remaining values were again obtained using interpolation. For the fourth column we simply assumed 18% prior IS use in the EEA/ROW instead of 23.5% (p 11), while for the fifth column, we work on the assumption of 58.4% JCV-seroprevalence (first- vs second-generation assay, see p 11).

		correct		
exposure	Bloomgren et al	55% JCV+, 18.69% prior IS	55% JCV+, 16% prior IS	58.4% JCV+, 16% prior IS
25+ months	4681	4681	4007	4255
26+ months	4681	4534	3881	4121
27+ months	4681	4388	3756	3988
28+ months	4681	4243	3632	3857
29+ months	4681	4099	3509	3726
30+ months	4681	3956	3386	3595
31+ months	4681	3815	3265	3467
32+ months	4681	3675	3145	3339
33+ months	4681	3535	3026	3213
34+ months	4681	3397	2908	3088
35+ months	4681	3260	2790	2962
36+ months	4681	3125	2674	2839
37+ months	4681	2990	2559	2717
38+ months	4681	2857	2445	2596
39+ months	4681	2724	2331	2475
40+ months	4681	2593	2219	2356
41+ months	4681	2463	2108	2238
42+ months	4681	2334	1998	2122
43+ months	4681	2206	1888	2005
44+ months	4681	2080	1780	1890
45+ months	4681	1954	1673	1776
46+ months	4681	1830	1566	1663
47+ months	4681	1707	1461	1551
48+ months	4681	1585	1356	1440
total	112 344	74 031	63 363	67 279

Table 13: Estimating the total exposure with JCV+, IS+ patients in the cohort from [Bloomgren et al 2012].

In the next table we carry out the same procedure, for both STRATA and post-marketing, with all patients having had 49–72 natalizumab infusions, using the data as of August 2013. The numbers for patients with either (exactly) 49, 54, 60, 61, 66 or 72 infusions are contained in [O'Connor et al 2014, figures e-2 and e-3]; the remaining ones

were once again obtained using interpolation. Note that, for STRATA, we apply the correction factor (1) from §2, to account for the higher JCV-seroprevalence and the lower prior IS use in STRATA (see the discussion on p 4):

	Biogen Idec method		correct	
infusions	post-marketing	STRATA	post-marketing	STRATA
49	29 197	724	29 197	724
50	29 197	724	28 147	716
51	29 197	724	27 073	707
52	29 197	724	25 986	697
53	29 197	724	24 898	686
54	29 197	724	23 818	680
55	29 197	724	22 758	675
56	29 197	724	21 729	673
57	29 197	724	20 743	671
58	29 197	724	19 810	667
59	29 197	724	18 943	663
60	29 197	724	18 152	655
61	29 197	724	17 392	644
62	29 197	724	16 456	632
63	29 197	724	15 350	623
64	29 197	724	14 144	615
65	29 197	724	12 911	607
66	29 197	724	11 723	602
67	29 197	724	10 638	597
68	29 197	724	9658	592
69	29 197	724	8772	585
70	29 197	724	7968	576
71	29 197	724	7235	563
72	29 197	724	6562	546
total	700 728	17 376	420 063	15 396

Table 14: Estimating the total exposure with the STRATA cohort [O'Connor et al 2014].

So in post-marketing, as of August 2013, just (approximately) 420 063 infusions had actually been given to patients in the period in question, not 700 728; similarly, in STRATA, the total experience equals about 15 396 infusions (not 17 376).

E The PML risk in STRATA estimated the actuarial way

In this section we carry out using the data from [O'Connor et al 2014] what we did in appendix B using the data from [Keller-Stanislawski 2011], ie, we compute the incidence of PML in STRATA the actuarial way (see §7 for a description of this method).

The labelling of the columns in the table below uses the same notation as table 11 in appendix B; the most important columns are the ones labelled 'cum. in.' (cumulative incidence), which give the incidence of PML during the first i natalizumab infusions in STRATA:

inf.	cases	patients	q_i	$q_1 q_2 \cdots q_i$	cum. in.	inf.	cases	patients	q_i	$q_1 q_2 \cdots q_i$	cum. in.
1	0	1087	1.000000	1.000000	0.00‰	43	0	670	1.000000	0.997201	2.80‰
2	0	1081	1.000000	1.000000	0.00‰	44	1	665	0.998496	0.995701	4.30‰
3	0	1074	1.000000	1.000000	0.00‰	45	0	660	1.000000	0.995701	4.30‰
4	0	1067	1.000000	1.000000	0.00‰	46	1	655	0.998473	0.994181	5.82‰
5	0	1061	1.000000	1.000000	0.00‰	47	0	651	1.000000	0.994181	5.82‰
6	0	1054	1.000000	1.000000	0.00‰	48	1	646	0.998452	0.992642	7.36‰
7	0	1031	1.000000	1.000000	0.00‰	49	0	641	1.000000	0.992642	7.36‰
8	0	993	1.000000	1.000000	0.00‰	50	1	634	0.998423	0.991076	8.92‰
9	0	949	1.000000	1.000000	0.00‰	51	1	626	0.998403	0.989493	10.51‰
10	0	907	1.000000	1.000000	0.00‰	52	0	617	1.000000	0.989493	10.51‰
11	0	873	1.000000	1.000000	0.00‰	53	0	608	1.000000	0.989493	10.51‰
12	0	855	1.000000	1.000000	0.00‰	54	0	602	1.000000	0.989493	10.51‰
13	0	854	1.000000	1.000000	0.00‰	55	0	598	1.000000	0.989493	10.51‰
14	0	847	1.000000	1.000000	0.00‰	56	0	596	1.000000	0.989493	10.51‰
15	0	832	1.000000	1.000000	0.00‰	57	1	594	0.998316	0.987827	12.17‰
16	0	812	1.000000	1.000000	0.00‰	58	0	591	1.000000	0.987827	12.17‰
17	0	791	1.000000	1.000000	0.00‰	59	0	587	1.000000	0.987827	12.17‰
18	0	774	1.000000	1.000000	0.00‰	60	0	580	1.000000	0.987827	12.17‰
19	0	763	1.000000	1.000000	0.00‰	61	1	570	0.998246	0.986094	13.91‰
20	0	758	1.000000	1.000000	0.00‰	62	0	560	1.000000	0.986094	13.91‰
21	0	756	1.000000	1.000000	0.00‰	63	0	552	1.000000	0.986094	13.91‰
22	0	756	1.000000	1.000000	0.00‰	64	0	545	1.000000	0.986094	13.91‰
23	0	755	1.000000	1.000000	0.00‰	65	0	538	1.000000	0.986094	13.91‰
24	0	752	1.000000	1.000000	0.00‰	66	0	533	1.000000	0.986094	13.91‰
25	0	746	1.000000	1.000000	0.00‰	67	0	529	1.000000	0.986094	13.91‰
26	0	740	1.000000	1.000000	0.00‰	68	0	524	1.000000	0.986094	13.91‰
27	0	735	1.000000	1.000000	0.00‰	69	0	518	1.000000	0.986094	13.91‰
28	0	731	1.000000	1.000000	0.00‰	70	0	510	1.000000	0.986094	13.91‰
29	0	727	1.000000	1.000000	0.00‰	71	1	499	0.997996	0.984118	15.88‰
30	0	724	1.000000	1.000000	0.00‰	72	1	484	0.997934	0.982085	17.92‰
31	0	721	1.000000	1.000000	0.00‰	73	0	465	1.000000	0.982085	17.92‰
32	0	718	1.000000	1.000000	0.00‰	74	0	447	1.000000	0.982085	17.92‰
33	1	715	0.998601	0.998601	1.40‰	75	0	432	1.000000	0.982085	17.92‰
34	1	713	0.998597	0.997201	2.80‰	76	1	416	0.997596	0.979724	20.28‰
35	0	709	1.000000	0.997201	2.80‰	77	0	400	1.000000	0.979724	20.28‰
36	0	706	1.000000	0.997201	2.80‰	78	1	382	0.997382	0.977159	22.84‰
37	0	702	1.000000	0.997201	2.80‰	79	0	360	1.000000	0.977159	22.84‰
38	0	697	1.000000	0.997201	2.80‰	80	0	334	1.000000	0.977159	22.84‰
39	0	692	1.000000	0.997201	2.80‰	81	0	301	1.000000	0.977159	22.84‰
40	0	686	1.000000	0.997201	2.80‰	82	0	262	1.000000	0.977159	22.84‰
41	0	681	1.000000	0.997201	2.80‰	83	0	213	1.000000	0.977159	22.84‰
42	0	675	1.000000	0.997201	2.80‰	84	0	155	1.000000	0.977159	22.84‰

Table 15: Actuarial risk of natalizumab-associated PML, estimated using data from the STRATA study as of August 2013.

So, for example, with 78 natalizumab infusions, the incidence of PML in STRATA was 22.8‰ (about 1 in 44). On recalling that only 67% of STRATA patients were JCV-seropositive, the cumulative incidence for the first six years of natalizumab treatment in this group was therefore approximately 34.0‰ (1 in 29).

F The average number of patient-months for the third year of natalizumab therapy

Worldwide natalizumab patient numbers were as follows (the data as of June 2010 are drawn from [Bozic et al 2010, figure 1]; for the June 2014 figures, see [TY-PAN-0597(16) 2014, slide 9]):

exposure	30th June 2010	30th June 2014
overall	71 400	129 100
12+ months	44 700	98 300
18+ months	34 800	85 500
24+ months	26 300	73 500
30+ months	15 800	62 300
36+ months	8600	53 300
42+ months	3500	44 700

Table 16: Global post-marketing natalizumab exposures as of mid-2010 vs mid-2014.

Using this information, the following estimates of the patient numbers for each month of the third year of natalizumab therapy were hence obtained through interpolation:

exposure	30th June 2010	30th June 2014
25+ months	24 611	71 537
26+ months	22 832	69 595
27+ months	21 011	67 687
28+ months	19 199	65 826
29+ months	17 445	64 026
30+ months	15 800	62 300
31+ months	14 302	60 656
32+ months	12 945	59 087
33+ months	11 713	57 579
34+ months	10 589	56 121
35+ months	9557	54 698
36+ months	8600	53 300
total	188 604	742 412

Table 17: Breakdown of the exposures for the third year of natalizumab therapy.

Therefore, as of 30th June 2010, the average number of patient-months with the third year of natalizumab therapy was $188\,604 \div 24\,611 = 7.7$, while as of 30th June 2014, that average was $742\,412 \div 71\,537 = 10.4$ (+35.1%).

The next table shows the distribution of all infusions administered in the third year of therapy across the three trimesters:

trimester	30th June 2010	30th June 2014
1	46.47% (87 653 of 188 604)	36.99% (274 645 of 742 412)
2	32.07% (60 492 of 188 604)	33.14% (246 069 of 742 412)
3	21.45% (40 459 of 188 604)	29.86% (221 698 of 742 412)

Table 18: Weight of each trimester of the third year of natalizumab therapy.

G Statistically significant evidence that risk-stratification works

In this appendix, we provide references for all figures used in §4, to back up our claim that risk-stratification really does work. The first table contains the reported number of PML cases at various times in the past.

date	no. of PML cases	reference
4th Jan. 2012	201	[Giovannoni 2012c]
2nd Jan. 2013	323	[Reder 2013]
3rd Sept. 2013	401	[TY-PAN-0597(4) 2013, slide 7]
2nd Sept. 2014	495	[TY-PAN-0597(16) 2014, slide 7]

Table 19: Total number of natalizumab-associated PML cases at various points in time.

The next table gives the total natalizumab exposure (patient-years), again for several points in time.

date	total natalizumab exposure (patient-years)	reference
31st Dec. 2011	195 500	[Giovannoni 2012d]
31st Dec. 2012	261 990	[TY-PAN-0587(1) 2013, slide 7]
30th Sep. 2013	313 560	[TY-PAN-0597(6) 2013, slide 8]
30th Sep. 2014	381 209	[TY-PAN-0597(17) 2014, slide 8]

Table 20: Total exposure in natalizumab-treated patients at various points in time.

In §4, we also make use of the fact that, as of 31st March 2011, the total post-marketing experience with months 25–36 of natalizumab treatment equalled 26 536 patient-years (37 860 as of 29th February 2012) and that, thus far, there had been 65 PML cases in this period (88 as of 29th February 2012 [Bloomgren et al 2012, figure 1]). To obtain the precise number of patient-years, we again perform interpolation. At the end of March 2011, worldwide patient numbers in the post-marketing setting only (ie, excluding clinical trials) were as follows [Kappos et al 2011a, p 746] (or [Bozic 2011, slide 5]): in total, 83 300 patients had been treated natalizumab, with 55 100 receiving the drug for at least 12 months, 44 900 for 18+ months, 35 400 for 24+ months, 27 400 for 30+ months, 18 700 for 36+ months, and 10 700 for 42+ months. At the end of February 2012, natalizumab post-marketing exposures were like so [Bloomgren et al 2012, figure 1]: of 99 571 patients treated with natalizumab, 65 981 had been on it for 13+ months, 45 533 for 25+ months, 29 085 for 37+ months, 14 239 for 49+ months, and 3596 for 61+ months. Interpolation then gives these figures:

	no. of patients	
months	31st March 2011	29th February 2012
25	33 999	45 533
26	32 655	44 091
27	31 344	42 665
28	30 047	41 254
29	28 739	39 858
30	27 400	38 476
31	26 013	37 106
32	24 585	35 747
33	23 127	34 398
34	21 652	33 059
35	20 172	31 727
36	18 700	30 403
total	318 433	454 317

Table 21: Global post-marketing exposures in the third year of natalizumab therapy as of 31st March 2011 vs 29th February 2012.

Hence, as of 31st March 2011, the total exposure for months 25–36 was equal to $318\,433 \div 12 = 26\,536$ years, while

as of 29th February 2012, this quantity was $454\,317 \div 12 = 37\,860$ years.

Moreover, from [Kappos et al 2011a, figure 1 (B)], as of 31st May 2011, the (Biogen Idec) incidence of PML for patients with 25–36 infusions was approximately 1.9‰; assuming it was the same two months earlier (a conservative estimate, because the incidence in question was still rising at that time), we deduce that, as of 31st March 2011, there were

$$33\,999 \times 1.9‰ = 65$$

PML cases in this category. Therefore, over the eleven-month period from the beginning of April 2011 until the end of February 2012, in the post-marketing setting, the number of PML cases in the third year of natalizumab therapy increased by $88 - 65 = 23$ while the exposure grew by $37\,860 - 26\,536 = 11\,324$ patient-years.

H Estimating the frequency of the previous immunosuppressant use

In the table below, we estimate the proportion of the prior use of immunosuppressants in the EEA/ROW as per §5 (p 10). The second column has the total number of PML cases as of July 2011 [Keller-Stanislawski 2011, slide 6] for each country with at least one case; the third column ('weight') states this as a percentage of all PML cases, ie, states the relative number of cases. (The rows are ordered by weight.) The fourth column contains the frequency of the former immunosuppressant use from TOP as of June 2011 [Kappos et al 2011b, figure 1] for the country in question as well as a corresponding 90% confidence interval; for those countries that do not participate in this trial—the starred ('$*$') ones—we assume a quarter prior immunosuppression. The right-most column finally contains the weighted upper endpoint of the confidence interval from the previous column, ie, the upper endpoint multiplied by the weight of the respective country; the sum of all values in this column then gives the weighted proportion of the prior immunosuppressive use in the EEA/ROW:

country	PML cases as of July 2011	weight	prior immunosuppressant use in TOP (proportion, 90% CI)	weight × upper endpoint of CI
Germany	27	31.40%	93 of 934 (9.96%, 8.39%–11.72%)	3.68%
France	12	13.95%	18 of 189 (9.52%, 6.25%–13.80%)	1.93%
Belgium/Switzerland†*	7	8.14%		2.03%
Greece	6	6.98%	15 of 106 (14.15%, 8.93%–20.95%)	1.46%
Italy	6	6.98%	60 of 279 (21.51%, 17.52%–25.95%)	1.81%
Spain	6	6.98%	5 of 63 (7.94%, 3.18%–15.97%)	1.11%
Netherlands	4	4.65%	12 of 214 (5.61%, 3.27%–8.93%)	0.42%
Sweden*	3	3.49%		0.87%
Austria*	2	2.33%		0.58%
Hungary*	2	2.33%		0.58%
Poland*	2	2.33%		0.58%
Portugal	2	2.33%	14 of 59 (23.73%, 14.95%–34.58%)	0.80%
Australia	1	1.16%	15 of 179 (8.38%, 5.24%–12.61%)	0.15%
Canada	1	1.16%	16 of 187 (8.56%, 5.44%–12.71%)	0.15%
Czech Republic	1	1.16%	172 of 349 (49.28%, 44.76%–53.82%)	0.63%
Iceland*	1	1.16%		0.29%
Ireland*	1	1.16%		0.29%
Luxemburg*	1	1.16%		0.29%
United Kingdom	1	1.16%	2 of 87 (2.30%, 0.41%–7.06%)	0.08%
total	86	100.00%		17.74%

Table 22: Estimating the prior immunosuppressant use with natalizumab-treated patients in the EEA/ROW from the data in the TOP study and the geographic distribution of PML cases as of July 2011.

†These countries are considered jointly. The reason is that in the chart [Keller-Stanislawski 2011, slide 6], the same colour is used for both countries, so that one cannot tell which PML case occurred in which of the two countries. Belgium actually is a member of TOP: as of June 2011, 39 of 434 (9.0%, 90% CI: 6.83%–11.57%) Belgian TOP subjects had had immunosuppression. On the other hand, Switzerland is not participating in TOP and thus assumed to have 25% prior immunosuppression. For computing the value in the fifth column, we used the higher of the two proportions—25% for Switzerland, 11.57% for Belgium—ie, with either country, we assumed 25% prior IS use.

I JCV-positivity with the original vs the second-generation ELISA

From [Lee et al 2013, table 4], with the second-generation JCV antibody assay, 97.0% of patients who had a positive test result with the original two-step ELISA remained positive, whereas 9.4% of samples tested negative with the latter turned out to be positive with the former. Hence the 54.7% JCV-seroprevalence from STRATIFY-2 (obtained using the 'first-generation' method) correspond to

$$54.7\% \times 97.0\% + 45.3\% \times 9.4\% = 57.3\%$$

with the second-generation assay; similarly the 57.1% from JEMS correspond to

$$57.1\% \times 97.0\% + 42.9\% \times 9.4\% = 59.4\%.$$

Applying the exact same formula separately to the respective proportions for each of the ten participating countries in JEMS (see [Bozic et al 2014, figure 1]) yields the following table:

	JCV seroprevalence in JEMS	
country	original two-step ELISA	second-generation assay
Australia	48.6%	52.0%
Austria	66.7%	67.8%
Belgium	54.4%	57.1%
Canada	56.3%	58.7%
Germany	61.0%	62.8%
Ireland	51.0%	54.1%
Netherlands	66.2%	67.4%
Portugal	69.5%	70.3%
Switzerland	55.6%	58.1%
United Kingdom	48.8%	52.1%

Table 23: Estimated JCV-seroprevalence in JEMS with the second-generation assay.

J Detailed explanation of why the dropout rate is correct

The following data on the post-marketing exposure of natalizumab were used (see table 6 in §5):

	Post-marketing exposure (patient numbers)					
date	overall	12+ mo.	24+ mo.	36+ mo.	48+ mo.	reference
31st Mar. '10	67 700	41 000	21 300	5 800		[Biogen Idec 2010, slide 18]
31st Mar. '11	83 300	55 100	35 400	18 700		[Bozic 2011, slide 5]
31st Mar. '12	99 600	68 700	47 600	30 600		[TY-PAN-0463q 2012, slide 9]
31st Mar. '13	115 400	83 000	59 100	41 100	26 600	[TY-PAN-0597(2) 2013, slide 9]
31st Mar. '14	125 800	95 900	70 600	50 700	35 300	[TY-PAN-0597(13) 2014, slide 8]

Table 24: Post-marketing natalizumab exposure at various times in the past.

We will now verify that $\frac{10\,500-8\,700}{10\,500} = 17.1\%$ reflects the dropout rate during the fourth treatment year between 1st April 2013 and 31st March 2014, as claimed. Suppose therefore that a patient was due to finish their fourth year of natalizumab therapy in the 12 months from 1st April 2013 until 31st March 2014. This patient must have been among those 41 100 who had had at least 36 months of exposure as of 31st March 2013; if not, the patient could not possibly complete four years of therapy on or before 31st March 2014. On the other hand, said patient was not among the 30 600 who had already been 36 months on natalizumab by 31st March 2012, as if that had been the case, then the patient would have finished their fourth year before 1st April 2013 in contradiction to what we assumed in the beginning. If that patient actually continued natalizumab treatment until the end of the fourth year (at least), then, by a similar argument, this patient was among the 8700 patients who had had at least 48 months of

natalizumab exposure on 31st March 2014 but not yet on 31st March 2013; if, however, the patient quit natalizumab sometime during the fourth year, then they cannot have been in this group of 8700 patients—they never reached the 48th month, and in particular not in the 12-month period under consideration—and therefore they must have been one of the 1800 patients in the numerator above. This shows why this fraction is equal to the proportion of dropouts for the fourth treatment year of natalizumab therapy in patients 'scheduled' to complete their fourth year between 1st April 2013 and 31st March 2014.

K Taking deriskification (risk-stratification) into account when estimating the risk of PML in JCV-positive patients with prior IS

In this section, we supply the missing details and calculations for what we said in §5 from p 14.

We will begin by computing how many individuals with at least two years of natalizumab exposure are still on therapy after a further i months, $i = 1, 2, \ldots, 11$, assuming that deriskification takes place as described in §5, for each of three categories (JCV+ with prior IS, JCV+ without prior IS, JCV−). We do this simply by starting out assuming that there are a hypothetical 1 000 000 natalizumab-treated patients, with 58.4% JCV-positive and 16% having had prior immunosuppressive treatment, and use the dropout proportions from table 4 to determine how many individuals in each of the three aforementioned groups are still taking the drug after i months. This will allow us to estimate the proportion of patients carrying the various combinations of risk factors, and hence the risk of PML in JCV-positive patients with prior IS. The first thing we will need are the monthly *continuation* rates that correspond to the annual dropout rates from table 4 (if p is the annual dropout rate for a certain constellation, then $1 - p$ is the annual continuation rate and $\sqrt[12]{1-p}$ is the respective monthly continuation rate):

risk constellation	annual dropout rate	annual continuation rate	monthly continuation rate
JCV+, prior IS	36.8%	63.2%	96.2541%
JCV+, no prior IS	16.6%	83.4%	98.5013%
JCV−	6.4%	93.6%	99.4546%

Table 25: Monthly continuation rates according to PML risk factors.

With these, it is now easy to estimate how many patients are still on the drug after i months. For example, if there are 1 000 000 natalizumab-treated patients at first, then 93 440 are JCV-positive and have had prior immunosuppression (because $58.4\% \times 16\% = 9.344\%$). Of these, 83 328 are still taking the drug after three months, since $93\,340 \times 0.962541^3 = 83\,328$. Similarly, of the initially 490 560 JCV-positive patients without previous immunosuppressive therapy, three months later, 468 833 are still on the drug.[28] Thus we obtain the following table:

	JCV−		JCV+, no prior IS		JCV+, prior IS	
month	no. of pts.	proportion	no. of pts.	proportion	no. of pts.	proportion
0	416 000	41.6000%	490 560	49.0560%	93 440	9.3440%
1	413 731	41.9232%	483 208	48.9632%	89 940	9.1136%
2	411 475	42.2454%	475 966	48.8666%	86 571	8.8881%
3	409 231	42.5665%	468 833	48.7661%	83 328	8.6674%
4	406 999	42.8866%	461 807	48.6618%	80 206	8.4516%
5	404 779	43.2056%	454 886	48.5539%	77 202	8.2404%
6	402 572	43.5236%	448 069	48.4424%	74 310	8.0340%
7	400 376	43.8405%	441 353	48.3275%	71 527	7.8320%
8	398 193	44.1564%	434 739	48.2090%	68 847	7.6346%
9	396 021	44.4711%	428 224	48.0873%	66 268	7.4416%
10	393 861	44.7848%	421 806	47.9623%	63 786	7.2529%
11	391 713	45.0974%	415 484	47.8341%	61 397	7.0685%
average		43.5183%		48.4249%		8.0568%

Table 26: Development of the distribution of PML risk factors after two years of natalizumab treatment.

[28] Therefore, after three months, the prior immunosuppressant use among JCV-positive patients is 15.1% (83 328 of 552 161).

The ‘average’ in the last line of the table is simply the sum of all entries in a column except the first one, divided by 11 (ie, the arithmetic mean of the values for months 1–11). We may use this average as we assume that the individuals who reached a particular month of therapy between April 2011 and February 2012 ‘arrived’ uniformly. Eg, as we shall see below, 11 534 patients started the third year in the aforementioned period (ie, reached month 25 of treatment); hence we will suppose that $11\,534 \div 11 = 1049$ individuals did so in April 2011, 1049 in May 2011, and so forth.

As described on p 14 already, there is one other thing that needs to be accounted for, namely the fact that some of the patients in question were still in their second treatment year, for which we assume that no deriskification takes place. For instance, people who reached the 25th month of therapy between March 2011 and February 2012 spent only one month in the deriskification period (not between one and eleven months). Similarly, those who made it to the 26th month spent two months in this period, unless they did so in April 2011 and therefore had only one month to check out their JCV status.

In this fashion, we obtain the next table, whose last line shows the proportions of natalizumab-treated patients who are JCV-positive and have had prior immunosuppression, for each month of therapy during the period from April 2011 through February 2012 (the column for months 35–48 is omitted as it is identical to the last column of the preceding table):

	mo. 25	mo. 26	mo. 27	mo. 28	mo. 29	mo. 30	mo. 31	mo. 32	mo. 33	mo. 34
04/11	9.1136%	9.1136%	9.1136%	9.1136%	9.1136%	9.1136%	9.1136%	9.1136%	9.1136%	9.1136%
05/11	9.1136%	8.8881%	8.8881%	8.8881%	8.8881%	8.8881%	8.8881%	8.8881%	8.8881%	8.8881%
06/11	9.1136%	8.8881%	8.6674%	8.6674%	8.6674%	8.6674%	8.6674%	8.6674%	8.6674%	8.6674%
07/11	9.1136%	8.8881%	8.6674%	8.4516%	8.4516%	8.4516%	8.4516%	8.4516%	8.4516%	8.4516%
08/11	9.1136%	8.8881%	8.6674%	8.4516%	8.2404%	8.2404%	8.2404%	8.2404%	8.2404%	8.2404%
09/11	9.1136%	8.8881%	8.6674%	8.4516%	8.2404%	8.0340%	8.0340%	8.0340%	8.0340%	8.0340%
10/11	9.1136%	8.8881%	8.6674%	8.4516%	8.2404%	8.0340%	7.8320%	7.8320%	7.8320%	7.8320%
11/11	9.1136%	8.8881%	8.6674%	8.4516%	8.2404%	8.0340%	7.8320%	7.6346%	7.6346%	7.6346%
12/11	9.1136%	8.8881%	8.6674%	8.4516%	8.2404%	8.0340%	7.8320%	7.6346%	7.4416%	7.4416%
01/12	9.1136%	8.8881%	8.6674%	8.4516%	8.2404%	8.0340%	7.8320%	7.6346%	7.4416%	7.2529%
02/12	9.1136%	8.8881%	8.6674%	8.4516%	8.2404%	8.0340%	7.8320%	7.6346%	7.4416%	7.2529%
avg.	9.1136%	8.9086%	8.7280%	8.5711%	8.4367%	8.3241%	8.2323%	8.1605%	8.1079%	8.0735%

Table 27: Proportions of JCV-positive natalizumab-treated patients with prior immunosuppression.

We are now in a position to put together the table that finally allows us to estimate the PML incidence for months 25–48 of natalizumab therapy in JCV-positive patients with prior immunosuppressive treatment. The post-marketing patient numbers as of 31st March 2011 and 29th February 2012 were obtained from the same sources as in appendix G (see the paragraph right before table 21); the next column is then simply the difference between these two, ie, the number of natalizumab-treated patients who joined the respective month of therapy between 1st April 2011 and 29th February 2012 (the ‘new’ patients). The fifth column is really just the transpose of the last row from the previous table (which gives the proportions of JCV-positive patients with previous immunosuppression among all ‘new’ patients).

The last column is then calculated by taking the respective value of the third column multiplied by 9.344% (as up to the end of March 2011, $58.4\% \times 16\% = 9.344\%$ of all patients are assumed to have been JCV-positive with prior immunosuppression, regardless of natalizumab exposure) plus the value in the fourth column (the number of ‘new’ patients) multiplied by the proportion in the fifth column (the fraction of ‘new’ patients who are JCV-positive and have had prior immunosuppression).

For example, there were 38 476 patients with 30 or more months of exposure, of whom 11 076 were ‘new’ (hence 27 400 ‘old’ patients). Thus, in total there were

$$27\,400 \times 9.344\% + 11\,076 \times 8.3241\% = 3482$$

JCV-positive patients with prior immunosuppressive treatment in the cohort from [Bloomgren et al 2012] who had had at least 30 months of natalizumab therapy.

Since the post-marketing patient numbers as of 31st March 2011 (see [Kappos et al 2011a, p 746] or [Bozic 2011, slide 5]) are only given for up to 42 months of natalizumab therapy, beyond that, we assumed that there were always 11 000 ‘new’ patients over the period in question:

exposure (months)	no. of patients as of 29th Feb. '12	31st Mar. '11	'new' pts. from Apr. '11–Feb. '12	prop. of ('new') JCV+ pts. w. prior IS	total JCV+ pts. w. prior IS
25+	45 533	33 999	11 534	9.1136%	4228
26+	44 091	32 655	11 436	8.9086%	4070
27+	42 665	31 344	11 320	8.7280%	3917
28+	41 254	30 047	11 208	8.5711%	3768
29+	39 858	28 739	11 119	8.4367%	3623
30+	38 476	27 400	11 076	8.3241%	3482
31+	37 106	26 013	11 093	8.2323%	3344
32+	35 747	24 585	11 162	8.1605%	3208
33+	34 398	23 127	11 271	8.1079%	3075
34+	33 059	21 652	11 407	8.0735%	2944
35+	31 727	20 172	11 555	8.0568%	2816
36+	30 403	18 700	11 703	8.0568%	2690
37+	29 085	17 247	11 838	8.0568%	2565
38+	27 772	15 826	11 947	8.0568%	2441
39+	26 467	14 448	12 019	8.0568%	2318
40+	25 170	13 127	12 043	8.0568%	2197
41+	23 884	11 873	12 011	8.0568%	2077
42+	22 612	10 700	11 912	8.0568%	1960
43+	21 354		11 000	8.0568%	1854
44+	20 114		11 000	8.0568%	1738
45+	18 893		11 000	8.0568%	1624
46+	17 693		11 000	8.0568%	1512
47+	16 516		11 000	8.0568%	1402
48+	15 364		11 000	8.0568%	1294
total					64 147

Table 28: Total natalizumab exposure in JCV-seropositive post-marketing patients with prior immunosuppression in the cohort from Bloomgren et al.

L Estimating the risk of PML in JCV-positive patients without prior IS for months 49–72 of natalizumab treatment

In §6, we claim that as of 5th March 2013, there must have been 62 to 66 PML cases in JCV-positive patients without prior immunosuppressive treatment during month 49–72 of natalizumab therapy in the post-marketing setting. This information was obtained by 'reverse-engineering' the respective 95% confidence interval, which is

$$4.8\text{–}7.8‰,$$

see [TY-PAN-0597(17) 2014, slide 9]. We proceeded as follows. Since we know that the incidence was 6.1‰, we can simply try various combinations of m and n such that

$$\frac{m}{n} \times 1000 = 6.1$$

and see what a 95% confidence interval looks like in each case. (Note that $n \approx 163.9 \times m$ always.) We found that for $m = 62, 63, 64, 65, 66$, the resulting 95% confidence interval is exactly as above; already if $m = 61$ or $m = 67$, however, the interval is too wide respectively too narrow. (These confidence intervals were all calculated using the *modified Wald method*.)

The next table shows how to compute the true proportion (ie, compared to what Biogen Idec assume) of infusions that were actually given in patients with 49–72 months of natalizumab treatment before March 2013, again by performing interpolation, on the patient numbers from [Bloomgren et al 2012, figure 1]. As explained, we technically estimate said proportion with all infusions administered prior to March 2012 in those with 37–60 months of therapy (see p 15 in §6), which is fully sufficient since we may assume that the dropout rate in this group for the following 12-month period (whatever it turned out to be) was uniform:

	patient numbers	
exposure	Biogen Idec	correct
37+ months	29 085	29 085
38+ months	29 085	27 687
39+ months	29 085	26 319
40+ months	29 085	24 979
41+ months	29 085	23 669
42+ months	29 085	22 388
43+ months	29 085	21 137
44+ months	29 085	19 914
45+ months	29 085	18 721
46+ months	29 085	17 556
47+ months	29 085	16 421
48+ months	29 085	15 316
49+ months	29 085	14 239
50+ months	29 085	13 192
51+ months	29 085	12 173
52+ months	29 085	11 184
53+ months	29 085	10 224
54+ months	29 085	9294
55+ months	29 085	8392
56+ months	29 085	7520
57+ months	29 085	6677
58+ months	29 085	5863
59+ months	29 085	5078
60+ months	29 085	4322
total	698 040	371 350

Table 29: Estimating the total exposure with JCV-positive patients without prior immunosuppression.

So only $371\,350 \div 698\,040 = 53.2\%$ of infusions were actually given.

M Taking deriskification (risk-stratification) into account when estimating the risk of PML in JCV-positive patients without prior IS

This section is very similar to the one for patients with prior immunosuppression (appendix K). However, we will also need the proportions of patients after i months of deriskification as per table 25 who are JCV-positive but have not used immunosuppressants previously, $i = 1, 2, \ldots, 24$ (cf table 26, which goes out to $i = 11$ only):

	JCV−		JCV+, no prior IS		JCV+, prior IS	
month	no. of pts.	proportion	no. of pts.	proportion	no. of pts.	proportion
12	389 577	45.4089%	409 258	47.7029%	59 097	6.8883%
13	387 452	45.7193%	403 124	47.5686%	56 883	6.7122%
14	385 339	46.0286%	397 083	47.4313%	54 752	6.5401%
15	383 238	46.3368%	391 132	47.2912%	52 701	6.3720%
16	381 147	46.6438%	385 270	47.1483%	50 727	6.2078%
17	379 069	46.9498%	379 496	47.0027%	48 827	6.0475%
18	377 001	47.2547%	373 808	46.8544%	46 998	5.8909%
19	374 945	47.5584%	368 206	46.7036%	45 237	5.7379%
20	372 901	47.8611%	362 688	46.5503%	43 543	5.5886%
21	370 867	48.1626%	357 252	46.3946%	41 912	5.4429%
22	368 844	48.4630%	351 898	46.2365%	40 342	5.3006%
23	366 833	48.7623%	346 625	46.0761%	38 831	5.1617%
24	364 832	49.0604%	341 430	45.9135%	37 376	5.0261%

Table 30: Continuation of table 26 for up to 24 months.

The next table is the no-prior-IS equivalent of table 28 (using patient numbers as of 31st March 2013 instead of 29th February 2012, see [TY-PAN-0597(2) 2013, slide 9]):

exposure	no. of patients as of 31st Mar. '13	31st Mar. '11	'new' pts. from Apr. '11–Mar. '13	prop. of ('new') JCV+ pts. w.o. prior IS	total JCV+ pts. w.o. prior IS
25+ months	57 359	33 999	23 360	48.9632%	28 116
26+ months	55 642	32 655	22 987	48.8706%	27 253
27+ months	53 959	31 344	22 615	48.7785%	26 407
28+ months	52 318	30 047	22 271	48.6873%	25 583
29+ months	50 728	28 739	21 989	48.5974%	24 784
30+ months	49 200	27 400	21 800	48.5091%	24 016
31+ months	47 739	26 013	21 726	48.4228%	23 281
32+ months	46 337	24 585	21 752	48.3390%	22 575
33+ months	44 983	23 127	21 856	48.2578%	21 892
34+ months	43 666	21 652	22 014	48.1797%	21 228
35+ months	42 376	20 172	22 204	48.1049%	20 577
36+ months	41 100	18 700	22 400	48.0338%	19 933
37+ months	39 830	17 247	22 583	47.9667%	19 293
38+ months	38 567	15 826	22 741	47.9038%	18 657
39+ months	37 310	14 448	22 862	47.8454%	18 026
40+ months	36 062	13 127	22 935	47.7918%	17 401
41+ months	34 825	11 873	22 952	47.7433%	16 782
42+ months	33 600	10 700	22 900	47.7000%	16 172
43+ months	32 389		20 000	47.6623%	15 610
44+ months	31 193		20 000	47.6304%	15 017
45+ months	30 015		20 000	47.6044%	14 434
46+ months	28 856		20 000	47.5847%	13 861
47+ months	27 717		20 000	47.5713%	13 300
48+ months	26 600		20 000	47.5645%	12 751
total					476 949

Table 31: Exposure in JCV-positive post-marketing natalizumab patients without prior immunosuppression as of 31st Mar. '13.

Some explanations.

The second column was obtained from the data in [TY-PAN-0597(2) 2013, slide 9] and again using interpolation. The third column is the same as in table 28. The fourth column (the number of 'new' patients) is then simply the difference of the second and third columns; since the values in the third column go out to month 42 only, we assumed that there were 20 000 'new' patients with 43+ months of exposure.

The fifth column was obtained as follows. Suppose that the proportions in table 26 joined with table 30 in the third column from the right (ie, for JCV+ patients without prior IS) are labelled $p_1, p_2, \ldots, p_{24}$. Then the fifth column of table 31 was obtained like so. For month 48, we just took the average of all the p's, ie,

$$\frac{p_1 + p_2 + \cdots + p_{24}}{24}.$$

The reason is that we again assume that the 'new' patients arrived at uniform speed, ie, $20\,000 \div 24$ per month. For month 47, the proportion is

$$\frac{p_1 + p_2 + \cdots + 2p_{23}}{24}.$$

Here, p_{23} is used twice while p_{24} is omitted, which is since patients spent at most 23 months in the deriskifcation period (ie, beyond the second year of natalizumab therapy). Continuing in this way, for month 26 the proportion is

$$\frac{p_1 + 23p_2}{24},$$

because those who reached month 26 in April 2011 had only one month to check out their JCV status, while everyone else had two months.

Finally, the values in the right-most column of table 31 were then computed by taking the respective value in the third column multiplied by $58.4\% \times 84\%$ (58.4% JCV+ with 84% having had no prior immunosuppression) plus the value in the fourth column multiplied by the proportion in the fifth column. This gives an accurate estimate of the total number of JCV-positive patients without prior immunosuppression, as it assumes that there was no deriskification prior to the introduction of JCV testing.

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